GR

ORIGINAL SUBMISSION

000001

GRAS ASSOCIATES, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com

July 7, 2011

Food and Drug Administration
Center for Food Safety & Applied Nutrition
Office of Food Additive Safety (HFS-255)
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Dr. Mary D. Ditto

Re: GRAS Notification – High Purity Rebaudioside A (≥ 95%)

Dear Dr. Ditto:

On behalf of Compound Solutions, Inc. of Vista, CA, we are submitting for FDA review a GRAS notification for high purity Rebaudioside A (≥ 95%). The attached documentation contains the specific information that addresses the safe human food uses for the subject notified substance as discussed in the GRAS guidance document.

If additional information or clarification is needed as you and your colleagues proceed with the review, please feel free to contact me via telephone or email.

We look forward to your feedback.

Sincerely,

(b) (6)

Robert S. McQuate, Ph.D.
CEO & Co-Founder
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074
541-678-5522
mcquate@gras-associates.com
www.gras-associates.com

Enclosure: GRAS Notification for Compound Solutions, Inc. – High Purity Rebaudioside A (≥ 95%) (in triplicate)

000002

GRAS ASSOCIATES, LLC

GRAS ASSESSMENT

HIGH PURITY REBAUDIOSIDE A (≥ 95%)

Food Usage Conditions for General Recognition of Safety

for

Compound Solutions, Inc.
Vista, CA

Evaluation by

Richard C. Kraska, Ph.D., DABT
Robert S. McQuate, Ph.D.
Madhusudan G. Soni, Ph.D., FACN

July 5, 2011

TABLE OF CONTENTS

TABLE OF CONTENTS continued

TABLES

FIGURES

APPENDICES

I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)[1]

Compound Solutions, Inc. ("Compound Solutions") has determined that its high purity rebaudioside A (≥ 95%) product, which is referred to as ViaSweet® and which meets the specifications as described below, is Generally Recognized As Safe (GRAS) in accordance with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination was made in concert with an appropriately convened panel of experts who are qualified by scientific training and experience. The GRAS determination is based on scientific procedures as described in the following sections. The evaluation accurately reflects the conditions of the stevia-derived sweetener's intended uses in foods.

Signed:

(b) (6)

July 6, 2011

Robert S. McQuate, Ph.D.
GRAS Associates, LLC
20482 Jacklight Lane
Bend, OR 97702-3074

Date

B. Name & Address of Notifier

Compound Solutions, Inc.
2350 Oak Ridge Way
Vista, CA 92081

As the notifier, Compound Solutions accepts responsibility for the GRAS determination that has been made for its purified rebaudioside A product[2] as described in the subject notification; consequently, these rebaudioside A preparations, i.e., having purities of no less than 95% rebaudioside A, that meet the conditions described herein are exempt from pre-market approval requirements for food ingredients.

[1] See 62 FR 18938 (17 April 1997). http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

[2] Compound Solutions refers to its high purity rebaudioside A product from leaves of *Stevia rebaudiana* Bertoni with the trade name of ViaSweet®.

C. Common Name & Identity of Notified Substance

High purity rebaudioside A, generally abbreviated as reb A or Reb A, is the common name for the notified substance; also see Section III.A.

D. Conditions of Intended Uses in Food

The high purity rebaudioside A preparations are intended to be used as a table top sweetener and as a general purpose non-nutritive sweetener for incorporation into foods in general, other than in meat and poultry products, at per serving levels that reflect good manufacturing practices principles in that the quantity added to foods should not exceed the amount reasonably required to accomplish its intended technical effect.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, Compound Solutions' high purity rebaudioside A (≥ 95%) preparations from the leaves of *Stevia rebaudiana* Bertoni have been determined to be GRAS on the basis of scientific procedures as discussed in the detailed description provided below.

F. Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to the US Food and Drug Administration (FDA) upon request or will be available for review and copying at reasonable times at the offices of GRAS Associates, LLC, located at 20482 Jacklight Lane, Bend, OR 97702-3074.

II. INTRODUCTION

A. Objective

At the request of Compound Solutions, GRAS Associates, LLC ("GA") has undertaken an independent safety evaluation of its ViaSweet® preparations. The preparations consist of high purity rebaudioside A that is extracted from the leaves of *Stevia rebaudiana* Bertoni and purified to yield rebaudioside A with a purity of ≥ 95%. The purpose of the evaluation is to ascertain whether or not the intended food uses of the subject high purity rebaudioside A as a non-nutritive general purpose sweetener as described in Section IV.A are generally recognized as safe, i.e., GRAS.

B. Foreword

Compound Solutions provided GA with background information needed to enable the GRAS assessment to be undertaken. In particular, the information that was provided addressed the safety/toxicity of steviol glycosides; the history of use of stevia in food; and compositional details, specifications, and method of preparation of its purified rebaudioside A. Compound Solutions was asked to provide adverse reports, as well as those that supported conclusions of safety. Safety/toxicity studies performed with animals were noted to have value, along with available human testing. Compound Solutions was also asked to supply past and present human food use information. Knowing how much steviol glycosides has been safely consumed, i.e., the use levels, is critical in extrapolating to safe exposures for rebaudioside A when consumed as a food ingredient. The composite safety/toxicity studies, in concert with exposure information, ultimately provide the specific scientific foundation for the GRAS determination.

Compound Solutions provided the product specifications, chemical properties, manufacturing, and safety related information. Compound Solutions also provided some consumption/ exposure information, along with other related documentation. This was augmented with an independent search of the scientific and regulatory literature extending through June 30, 2011. A GRAS assessment based on the composite safety information, i.e., based on scientific procedures, was undertaken. Those references that were deemed pertinent to the objective at hand are listed in Section VIII.

C. Summary of Regulatory History of Stevia

Stevia derived sweeteners are permitted as food additives in South America and in several countries in Asia, including China, Japan, and Korea. In recent years, these sweeteners have received food usage approvals in Mexico, Australia, New Zealand, Switzerland, France and Hong Kong. In the US, steviol glycosides have been used as a dietary supplement since 1995 (Geuns, 2003). The available information from US FDA dockets did not reveal any New Dietary Ingredient Notification for uses of purified rebaudioside. Between 1989 and 2008, at least two GRAS

petitions seeking authorization for the addition of stevioside or steviol glycosides to foods had been submitted to FDA. However, no authorizations had been issued by FDA in response to these filings, and these petitions were withdrawn. It appears that the previously available safety data---including purity considerations---for stevia, stevioside, or steviol glycosides were inadequate.

Based on available information from FDA's GRAS Notice Inventory[3] website as of July 5, 2011, the agency has received 19 notices on rebaudioside A or steviol glycosides. Eleven of these notices have received "no questions" letters from the FDA, while eight notices remain under agency review. In May 2008, Merisant and Cargill independently submitted GRAS notifications for rebaudioside A, highly purified forms of the steviol glycosides, to FDA. On December 17, 2008, FDA issued "no questions" letters for each of these GRAS notices. Since December 2008, a series of GRAS notifications were submitted to FDA for stevia-derived sweetener products by the following companies: McNeil Nutritionals, LLC; Blue California; Sweet Green Fields, LLC; Wisdom Natural Brands; Sunwin and Wild Flavors (two notifications); Pyure Brands, LLC, PureCircle USA, Inc, and GLG Life Tech, Ltd. Each of these firms received a "no questions" letter from FDA.[4] Additionally, 8 notifications submitted to FDA by different manufacturers are pending with the agency.

The Joint Expert Committee on Food Additives (JECFA) has reviewed steviol glycosides at its 51st, 63rd, 68th and 73rd meetings. In 2000, JECFA published the original review on steviol glycosides (WHO, 2000). JECFA established a temporary ADI (acceptable daily intake) of 0-2 mg/kg (on a steviol basis) at its 63rd meeting (WHO, 2006). Additionally, JECFA finalized food grade specifications (FAO, 2007a), although they were subsequently updated in 2008 (FAO, 2008) and 2010 (FAO, 2010) (see below). At the 69th meeting, the temporary status of the ADI was removed, and the ADI was raised to 0-4 mg/kg bw/day (on a steviol basis) as a result of the JECFA review of recently completed clinical studies with steviol glycosides (WHO, 2008). In 2009, JECFA published a final monograph addendum on steviol glycosides (WHO, 2009).

In early 2009, a number of parties, including the government of Australia and the Calorie Control Council, submitted a request to the Codex Committee on Food Additives in which it was proposed that the JECFA specifications for steviol glycosides should be modified to allow inclusion of Rebaudioside D and Rebaudioside F as specifically named acceptable glycosides that would be considered as part of the minimum 95% steviol glycosides composition (CCFA, 2009). This proposed modification was endorsed by the Codex Alimentarius Committee in July 2009; it was on the agenda for discussion at the JECFA Meeting in June, 2010 (WHO, 2009), and JECFA recently took final action in approving the modified steviol glycosides specifications to include Rebaudioside D and Rebaudioside F (FAO, 2010; Appendix A).

---

[3] Accessible at: http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=grasListing&displayAll=true.

[4] GRAS notification 252 was submitted by Merisant, GRAS notification 253 was submitted by Cargill, GRAS notification 275 was submitted by McNeil Nutritionals, GRAS notification 278 was submitted by Blue California, GRAS notification 282 was submitted by Sweet Green Fields, GRAS notification 287 was submitted by Wisdom Natural Brands, GRAS notifications 303 and 304 were submitted by Sunwin and Wild Flavors, GRAS notification 318 was submitted by Pyure Brands, GRAS notification 323 was submitted by PureCircle USA, and GRAS notification 329 was submitted by GLG Life Tech; information pertaining to these notifications are listed on FDA's website at http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing, along with their respective "no questions" letters.

In 2008, Switzerland's Federal Office for Public Health (2008) approved the use of stevia as a sweetener citing the favorable actions of JECFA. Subsequently, France published its approval for the food uses of rebaudioside A with a purity of 97% (AFSSA, 2009).

Also in 2008, the Food Standards Australia New Zealand (FSANZ) completed its evaluation of an application for use of steviol glycosides in foods. FSANZ recommended that the Australia and New Zealand Food Regulation Ministerial Council (Ministerial Council) amend the Australia New Zealand Food Standards Code to allow the use of steviol glycosides in food (FSANZ, 2008).

Based on a review of the international regulation of *Stevia rebaudiana* and the clinical evidence for safety and efficacy, on September 18, 2009, the Natural Health Products Directorate, Health Canada (2009), adopted the following guidelines for the use of Stevia and steviol glycosides in Natural Health Products (NHPs). The revised recommendation for the maximum limit for steviol glycosides in NHPs is in accordance with the full ADI (acceptable daily intake) of 4 mg steviol/kg bw established by WHO (2008).

In light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission for European Food Safety Authority (EFSA) to deliver a scientific opinion on the safety of steviol glycosides as a sweetener for use in the food categories specified in the dossiers from three petitioners, EFSA reexamined the safety of steviol glycosides (EFSA, 2010). After considering all the data on stability, degradation products, metabolism and toxicology, the EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day, which is similar to JECFA's determination.[5]

As of May 2010, the government of Hong Kong amended its food regulations to allow the use of steviol glycosides as a permitted sweetener in foods (Hong Kong, 2010). This action followed in the aftermath of the detailed safety evaluation and favorable findings as reported by JECFA.

D. FDA Regulatory Framework

Since 1995, steviol glycosides (or stevioside) have been used in dietary supplements in the US (Geuns, 2003). These supplements are widely available to consumers in the US through retail outlets and Internet purchases (Al-Achi and Greenwood, 2000). According to FDA regulation of foods, dietary supplements cannot legally be added to conventional foods. In order for their uses in conventional foods, dietary supplements must undergo premarket approval by FDA as food additives or, alternatively, the ingredients must be determined to be generally recognized as safe (GRAS). The authority to make GRAS determinations is not restricted to FDA. In fact, GRAS

[5] From a historical perspective, it is noted that the UK's Advisory Committee on Novel Foods and Processes for the Ministry of Agriculture, Fisheries and Food on September 24, 1998 rejected an application for use of steviol glycosides as a sweetener in herbal teas because "the applicant had not provided all of the information necessary to enable an assessment to be made." http://archive.food.gov.uk/maff/archive/food/novel/980924.htm In 1999, the Scientific Committee on Food for the European Commission concluded that "there are no satisfactory data to support the safe use of these stevia plants and leaves" (European Commission, 1999a). In another opinion also dated June 17, 1999, the Committee also reiterated "its earlier opinion that stevioside is not acceptable as a sweetener on the presently available data" (European Commission, 1999b).

determinations may be provided by experts who are qualified by scientific training and experience to evaluate the safety of food and food ingredients under the intended conditions of use.[6]

In 1997, FDA altered the GRAS determination process by eliminating the formal GRAS petitioning process. At that time, the petitioning process was replaced with a notification procedure.[7] While outlining the necessary content to be considered in making a GRAS determination, FDA encouraged that such determinations be provided to FDA in the form of a notification. However, notifying FDA of such determinations is strictly voluntary.

[6] See 21 CFR 170.3(i)(3).
[7] See 62 FR 18938 (17 April 1997). http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

III. CHEMISTRY & MANUFACTURE OF COMPOUND SOLUTIONS' HIGH PURITY REBAUDIOSIDE A (≥ 95%)

A. Common or Usual Name

The common or usual name for the products that are the subject of this notification is high purity rebaudioside A, which is derived from the leaves of *Stevia rebaudiana* Bertoni. Rebaudioside A is one of the common steviol glycosides found in nature. The rebaudioside A content of the commercial product is equal to or higher than 95%. ViaSweet® is the commercial name used by Compound Solutions in referring to the notified substance. In the scientific literature, steviol glycosides have been referred to as stevia, stevioside, steviol glycosides, and stevia glycoside. JECFA adopted the term, steviol glycosides, for the family of steviol derivatives with sweetness properties that are derived from the stevia plant. Presently, the term, stevia, is used more narrowly to describe the plant or crude extracts of the plant, while reb A---like stevioside---is the common name for another one of the specific glycosides that is extracted from stevia leaves.

B. Description

In 2009, Food Chemicals Codex (FCC) has prepared a monograph with a description and specifications for rebaudioside A. As described in this monograph, rebaudioside A is a white to off-white, hygroscopic fine crystal, granule, or powder having a sweet taste (FCC, 2009). It is freely soluble in ethanol:water 50/50 (v/v) and is sparingly soluble in water and in ethanol. Rebaudioside A is obtained from the leaves of the *Stevia rebaudiana* Bertoni plant in a multistep separation and purification process. The principal steps of manufacturing include extraction of steviol glycosides from the leaves using an aqueous or aqueous alcoholic (ethanol or methanol) solvent, and purification of rebaudioside A from the resulting mixture of steviol glycosides by resin absorption followed by recrystallization from an aqueous or aqueous alcoholic (ethanol or methanol) solvent. It is primarily composed of rebaudioside A, a glycoside of the *ent*-kaurenoid diterpenoid aglycone known as steviol (FCC, 2009).

C. Chemistry of Rebaudioside A

At its 51st meeting JECFA reviewed the safety related information, including chemistry on stevioside. The following description is taken from the original JECFA monograph (WHO, 2000).

> Stevioside is a glycoside of the diterpene derivative steviol (ent-13-hydroxykaur-16-en-19-oic acid). Steviol glycosides are natural constituents of the plant *Stevia rebaudiana Bertoni*, belonging to the Compositae family. The leaves of *S. rebaudiana Bertoni* contain eight different steviol glycosides, the major constituent being stevioside (triglucosylated steviol), constituting about 5-10% in dry leaves. Other main constituents are rebaudioside A (tetraglucosylated steviol), rebaudioside C, and dulcoside A. *S. rebaudiana* is native to South America and has been used to sweeten beverages and food for several centuries. The plant has also been distributed to Southeast Asia. Stevioside has a sweetening potency 250-300 times that of sucrose and is stable to heat. In a 62-year-old sample from a herbarium, the intense sweetness of *S. rebaudiana* was conserved, indicating the stability of stevioside to drying, preservation, and storage (Soejarto et al., 1982; Hanson and De Oliveira, 1993).

Of the eight different steviol glycosides found in stevia extract, the two principal sweeteners have been identified as rebaudioside A and stevioside. The chemical identities and key chemical identifiers for these two major glycosides are summarized in Table 1. The chemical structure of rebaudioside A is presented in Figure 1.

Table 1. Chemical Identity of Rebaudioside A & Stevioside

REBAUDIOSIDE A	
Common name	Rebaudioside A
Chemical name	13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D- glucopyranosyl) oxy] kaur-16-en-18-oic acid, β-D- glucopyranosyl ester
Chemical formula	$C_{44}H_{70}O_{23}$
Formula weight	967.03
CAS Number	58543-16-1
STEVIOSIDE	
Common Name	Stevioside
Chemical name	13-[2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester
Chemical formula	$C_{38}H_{60}O_{18}$
Formula weight	804.88
CAS Number	57817-89-7

JECFA (FAO, 2007b) identified the sweetener components of stevia and updated the list of common glycosides and their chemical structures (Figure 2) that are slightly different than compounds shown in other older publications (Nanayakkara et al., 1987; Suttajit et al., 1993). The structures of the components of stevia glycosides were also described in reviews by Kinghorn and Soejarto (1985), Kennelly (2002), and Geuns (2003). Other substances that lack sweetness include the labdane diterpenes, triterpenes, sterols and flavonoid glycosides.

Figure 1. Chemical Structure of Rebaudioside A

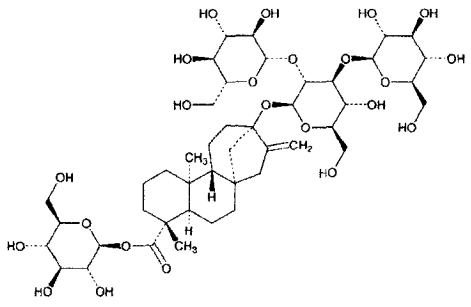
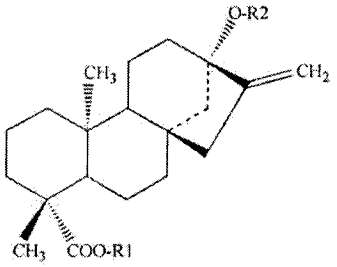


Figure 2. Chemical Structures of Various Steviol Glycosides Reproduced from FAO[a, b]



	Compound name	C.A.S. No.	R1	R2
1	Steviol	471-80-7	H	H
2	Steviolbioside	41093-60-1	H	β-Glc-β-Glc(2→1)
3	Stevioside	57817-89-7	β-Glc	β-Glc-β-Glc(2→1)
4	Rebaudioside A	58543-16-1	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
5	Rebaudioside B	58543-17-2	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
6	Rebaudioside C (dulcoside B)	63550-99-2	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
7	Rebaudioside D	63279-13-0	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
8	Rebaudioside E	63279-14-1	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
9	Rebaudioside F	438045-89-7	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
10	Rubusoside	63849-39-4	β-Glc	β-Glc
11	dulcoside A	64432-06-0	β-Glc	β-Glc-α-Rha(2→1)

[a] From FAO, 2007b.
[b] The indicated C.A.S. No. for Rubusoside as reported in the cited reference is incorrect and should be 64849-39-4.

D. Manufacturing Processes

Based on available scientific and patent literature, several manufacturing processes for steviol glycosides have been reported. These processes are summarized below, along with Compound Solutions' manufacturing process for its purified rebaudioside A (≥ 95%) preparation.

1. Scientific & Patent Literature

Generally, steviol glycosides are obtained by extracting leaves of *Stevia rebaudiana* Bertoni with hot water or alcohols (ethanol or methanol). The extract is a dark particulate solution containing all the active principles along with leaf pigments, soluble polysaccharides, and other impurities. Some processes remove the "grease" from the leaves with solvents such as chloroform or hexane before extraction occurs (Kinghorn and Soejarto, 1985). There are several extraction patents for the isolation of steviol glycosides. Kinghorn and Soejarto (1985) have categorized the extraction patents into those based on solvent, solvent plus a decolorizing agent, adsorption and column chromatography, ion exchange resin, and selective precipitation of individual glycosides. In recent patents, methods using ultrafiltration, metallic ions, supercritical fluid extraction with CO_2 and extract clarification with zeolite are employed.

At the 68th JECFA meeting, steviol glycosides were defined as the products obtained from the leaves of *Stevia rebaudiana* Bertoni. As described by JECFA, the typical manufacturing process starts with extracting leaves with hot water, and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides, and the product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product is commonly spray-dried.

2. Compound Solutions' Manufacturing Process for High Purity Rebaudioside A (≥ 95%)

The manufacturing process employed by Compound Solutions for its high purity rebaudioside A is fairly typical and similar to that yielding other related stevia-derived sweetener products on the market. The source of the Compound Solutions rebaudioside A preparations is the leaves of the *Stevia rebaudiana* (Bertoni) plant. Compound Solutions, in concert with Chengdu Wagott Pharmaceutical Company in China, has developed a state-of-the-art process for extracting rebaudioside A from the stevia leaf. The production of high purity rebaudioside A (≥ 95%) is carried out in two stages as summarized by flow diagrams in Appendix B-1 and B-2. In the first stage, the steviol glycosides primary extract containing 55% (± 5%) rebaudioside A is prepared, while in the second step the extract is further purified to obtain ≥ 95% rebaudioside A.

In the first step, dried/crushed leaves from selected varieties of *S. rebaudiana* (Bertoni) are extracted with water to obtain steviol glycosides. The extraction solution is filtered by ultra-filtration, the solids are discarded, and the filtrate is concentrated by reverse osmosis membrane separation. This is followed by adsorption onto polar resin, and the glycosides are subsequently eluted with methanol. The eluate is concentrated using an external circulation evaporator. The concentrate thus obtained is decolored with active carbon and filtered. The concentrate is spray dried or vacuum dried to obtain the primary steviol glycoside extracts rich in rebaudioside A (55 ± 5%)

The primary extract obtained above is further processed to obtain the high purity rebaudioside A. This process is shown in the flow diagram found in Appendix B-2. The stevia extract is dissolved in ethanol and water and filtered. Subsequently temperature controlled crystallization is carried out to obtain crude crystals containing ≥ 85% (± 5%) rebaudioside A. The crystals thus obtained

are rinsed with ethanol and spray dried. The crystallization and drying process is repeated one more time using ethanol to obtain high purity rebaudioside A (≥ 95%).

The ethanol and methanol used in the purification process comply with FCC 7th Edition specifications for these solvents. The ion exchange resins used in the manufacturing comply with 21 CFR 173.65. Compound Solutions' high purity rebaudioside A (≥ 95%) is prepared in accordance with current Good Manufacturing Practices (cGMP) at Chengdu Wagott Pharmaceutical Company facility: 5/F, A Building, Tianhe Incubator, Hi-Tech Incubating Park, Tianfu Ave., Chengdu, Sichuan 610041, China. In accordance with the policies set out in FDA's Compliance Policy Guide 110.310, Wagott's facility is registered with the FDA under registration number: 15414469282.

E. Product Specifications & Supporting Methods

1. JECFA Specifications for Steviol Glycosides

The composition of extracts of *Stevia rebaudiana* Bertoni depends upon the composition of the harvested leaves, which are, in turn, influenced by soil, climate, and the manufacturing process itself (FAO, 2007b).

In 2007, JECFA recommended that the method of assay should include a minimum requirement of 95% of the total of 7 specific steviol glycosides on a dried weight basis, and JECFA finalized food grade specifications at the 68th JECFA meeting with publication in the FAO JECFA Monograph 4 (FAO, 2007a). Stevioside and rebaudioside A are the major component glycosides of interest because of their sweetening property. The 5 other associated glycosides found in preparations of steviol glycosides accepted by the JECFA specifications with the 95% requirement are rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B. These, however, are typically found at much lower levels than stevioside or rebaudioside A. JECFA updated the specifications for steviol glycosides in 2008 (FAO, 2008), and then again in 2010 when the specifications were expanded to include the original seven specific steviol glycosides plus Reb D and Reb F (FAO, 2010); also see Appendix A.

Steviol glycosides are described as a white to yellow powder, odorless to having a slight characteristic odor, and exhibiting a sweetness that is 200-300 times greater than sucrose. The ingredient must consist of a minimum of 95% of 9 specific steviol glycosides. The steviol glycosides are freely soluble in water and ethanol, and the 1 in 100 solutions exhibit pH values between 4.5 - 7.0. The product should not have more than 1% ash with no more than a 6% loss on drying at 105°C for 2 hours. Any residual methanol levels should not exceed 200 ppm, and ethanol residues should not exceed 5000 ppm. Arsenic levels should not exceed 1 ppm as determined by the atomic absorption hydride technique. Lead levels should not exceed 1 ppm.

2. Specifications for Compound Solutions' High Purity Rebaudioside A (≥ 95%)

Wagott has adopted product specifications for its high purity rebaudioside A (≥ 95%) that meet or exceed JECFA recommendations while also complying with Food Chemicals Codex (FCC,

2009) specifications for rebaudioside A. A comparison of the specifications provided by Compound Solutions and those from JECFA and FCC is presented in Table 2. Batch analysis data from five lots demonstrating that ViaSweet® meets the required specifications are provided in Appendix C-1. An analytical report related to the identity of the ingredients from multiple batches of ViaSweet®, along with details of the methodology, is included as Appendix C-2 Part 1. This Appendix (C-2 Part-1) also includes residual solvent levels from five bathes and the pesticide residue analysis test report from one batch. In addition to this, Appendix C-2 Part-2 includes embedded Appendices 1 through 11 that provide details of:

- Analytical method
- Calibration
- Selectivity (Solvent Blank)
- Accuracy/Selectivity (Positive Control)
- Retention Time Markers
- Reference Standards System Suitability
- Sample Suitability
- Reference Standards System Suitability (USP Tailing Factor, Theoretical plates)
- Accuracy
- Repeatability, Precision, Results (Sample Chromatogram)
- Rebaudioside D and F Chromatograms

In the above-described information, analyses of pesticide residues were conducted from one production batch that was screened for 339 pesticide residues. The multi-residue pesticide screens revealed no pesticide residues (the detection limits varied among different groups and among individual compounds within a group between 0.01 and 0.06 mg/kg) in the rebaudioside A product. The results of this investigation are presented in Appendix C-3.

The collection of these reports demonstrates that the substance is well characterized and meets the purity criteria.

F. Stability Data for Stevioside & Rebaudioside A

Kinghorn and Soejarto (1985) reported that stevioside is a stable molecule over the pH range 3-9 and can be heated at 100°C for 1 hour, but at pH levels greater than 9 under these conditions it rapidly decomposes. These investigators also speculated that at pH 10 steviolbioside would be the major decomposition product produced from stevioside by alkaline hydrolysis. In another study, Chang and Cook (1983) investigated the stability of pure stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages. Some degradation of each sweetening component after 2 months of storage at 37°C was noted. However, no significant change at room temperature or below following 5 months of storage of stevioside and 3 months of storage of rebaudioside A was noted. Exposure to 1 week of sunlight did not affect stevioside, but resulted in approximately 20% loss of rebaudioside A. Heating at 60°C for 6 days resulted in 0-6% loss of rebaudioside A.

In a series of studies, Merisant (2008) conducted stability testing on rebaudioside A (1) as a powder, (2) as a pure sweetener in solution, and (3) on both cola-type and citrus carbonated beverages. In these investigations no degradation was detected when the powder was stored at 105°C for 96 hours. It was concluded that the powder was stable when stored for 26 weeks at 40±2°C with relative humidity of 75±5%. Both published and unpublished testing results from Merisant revealed that rebaudioside A in carbonated citric acid beverages and phosphoric acid beverages did not significantly degrade during prolonged storage at refrigeration, normal ambient, or elevated ambient temperatures. Minimal loss of rebaudioside A was detected after storage at 60°C, with considerable degradation noted after 13 hours at 100°C for carbonated beverage solutions and pure sweetener solutions (Merisant, 2008).

Cargill (2008) also investigated stability of rebaudioside A as a powder under various storage conditions and under a range of pH and temperatures. Additionally, Cargill also investigated rebaudioside A stability in several representative food matrices at room temperature and elevated temperatures. Stability profiles were created for table top sweetener applications, mock beverages including cola, root beer and lemon-lime, thermally processed beverages, yogurt, and white cake. The results of stability testing revealed some degradation products that had not been detected in bulk rebaudioside A. These degradation products were structurally related to the steviol glycosides that are extracted from the leaves of *Stevia rebaudiana* Bertoni. All the degradation products were found to share the same steviol aglycone backbone structure as found in stevioside and rebaudioside A, but they differ by virtue of the glucose moities present. The results of stability testing revealed that rebaudioside A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, rebaudioside A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

Cargill (2008, published as Clos et al., 2008) also conducted photostability studies on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis. The authors stated that the observation of better stability than in the work by Chang and Cook (1983) is due to differences in analytical methods. From the stability testing reported, it was concluded that Reb A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

Table 2. Specifications for Compound Solutions' High Purity Rebaudioside A (≥ 95%)

PARAMETER	JECFA[a] SPECIFICATIONS STEVIOL GLYCOSIDES	FCC SPECIFICATIONS REBAUDIOSIDE A	COMPOUND SOLUTIONS SPECIFICATIONS REBAUDIOSIDE A (≥ 95%)	METHODS
Appearance	White to light yellow powder	White to off-white, hygroscopic fine crystal, granule, or powder	White powder	Visual
Sweetness	200-300 times sweeter than sucrose	NA	300-400 times sweeter than sugar	Gustatory
Rebaudioside A	NA	NLT 95%	≥ 95%	HPLC
Total Steviol Glycosides	NLT 95%	NA	NA	HPLC
Other Related Steviol Glycosides (as Stev, Reb A, B, C, Dulc A, Rubu, and SB) on dry weight basis	NLT 95%	NMT 5%[b]	≤ 5%	HPLC
Moisture (loss on drying)	NMT 6%	NMT 6%	≤ 5%	CP[d] 2005
Ash	NMT 1%	NMT 1%	< 1%[C]	CP, 2005
Solubility	Freely soluble in water and ethanol	Freely soluble in water:ethanol (50:50)	Soluble in water	Visual
pH (1% solution)	4.5 - 7.0	4.5 - 7.0	4.5-7.0	
Residual solvent levels				
Residual Methanol	NMT 200 mg/kg	NMT 0.02%	< 200 mg/kg	GC
Residual Ethanol	NMT 5000 mg/kg	NMT 0.5%	< 5000 mg/kg	GC
Heavy metals				
Lead	NMT 1 mg/kg	NMT 1 mg/kg	< 1 mg/kg	CP, 2005
Arsenic	NMT 1 mg/kg	NMT 1 mg/kg	< 1 mg/kg	CP, 2005
Microbiological				
Total Plate Count	NA	NA	< 1000 cfu/g	CP, 2005
Total Yeast	NA	NA	≤ 100 cfu/g	CP, 2005
Total mold	NA	NA	≤ 100 cfu/g	CP, 2005
Coliform	NA	NA	Negative	CP, 2005
Escherichia coli	NA	NA	Negative	CP, 2005

[a] Prepared at 69th JECFA (2008). [b] Excludes Reb A but includes additional two glycosides Reb D and Reb F; [C] Sulfated ash; [d] Chinese Pharmacopeia; Abbreviations: St = Stevioside; Reb A = Rebaudioside A; Reb B = Rebaudioside B; Reb C = Rebaudioside C; Dulc A = Dulcoside A; Rub = Rubusoside; SB = Steviolbioside; NS = not specified; NA = not applicable; NLT = not less than; NMT = not more than.

Cargill (2008, published as Clos et al., 2008) also conducted photostability studies on the dry powder and mock beverages to ascertain Reb A behavior under defined conditions of fluorescent and near UV light exposure. Reb A was determined to be photostable under the defined conditions of analysis. The authors stated that the observation of better stability than in the work by Chang and Cook (1983) is due to differences in analytical methods. From the stability testing reported, it was concluded that Reb A is stable in various food matrices following several days or weeks of storage. The extent and rate of degradation is dependent on pH, temperature, and time. When placed in beverages, Reb A is more stable in the pH range 4 to 6 and at temperatures from 5°C to 25°C (Cargill, 2008).

In addition to the above described stability reports for purified rebaudioside A, in a GRAS notification by Sunwin (2010) on purified steviol glycosides with rebaudioside A and stevioside as the principal components, stability was investigated using a 0.04% solution of rebaudioside A 80% in acidic solutions between pH 2.81 and 4.18. In this study, the solutions were stored at 32°C for 4 weeks, and the Reb A content was determined at 1, 2 and 4 weeks. Rebaudioside A 80% was found to be very stable at pH 3.17 and above. At pH 2.81, after 4 weeks of storage under accelerated conditions only a 7% loss of Reb A was noted. Sunwin also studied the stability of rebaudioside A 80% in simulated beverages using 0.1 % citric acid (pH 3.2). The solutions were pasteurized and stored for 8 weeks at 4° and 32°C, and little difference in sweetness perception was found under these conditions.

Wagott on behalf of Compound Solutions also conducted stability testing on its rebaudioside A. In these studies, exposure to high illumination (4500 ± 500 lx), high temperature (40°C), and high humidity (90% RH) for 10 days showed no significant effects on content of rebaudioside A and total glycosides of the product. Accelerated testing of the product at the conditions of temperature (40°C ± 2°C) and humidity (75% ± 5% RH) for 6 months also did not show any significant changes in content of rebaudioside A and total glycosides (See Appendix D). The long term testing of the product at the conditions of temperature (25°C ± 2°C) and humidity (60% ± 10% RH) is ongoing, and results of the testing thus far completed for 18 months did not reveal any significant changes in the contents of rebaudioside A and total glycosides.

The available stability data in the scientific literature for rebaudioside A, the JECFA report, and the extensive stability testing presented by Merisant, Cargill and Sunwin, along with Compound Solutions' stability testing results, support the position that high purity rebaudioside A products are well-suited for the intended food uses as proposed by Compound Solutions.

IV. INTENDED DIETARY USES

A. Intended Uses

The subject Compound Solutions ViaSweet® preparations of high purity rebaudioside A (≥ 95%) are intended to be used as a table top sweetener and general purpose non-nutritive sweetener in various foods except meat and poultry products The intended use will be as a non-nutritive sweetener as defined in 21 CFR 170.3(o)(19)[8]. The intended use levels will vary by actual food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. However, the amounts of purified rebaudioside A (≥ 95%) to be added to foods will not exceed the amounts reasonably required to accomplish its intended technical effect in foods as required by FDA regulation.[9]

B. Food Uses As Addressed by JECFA, Merisant & Cargill

As part of its safety deliberations, JECFA reviewed various estimates of possible daily intake of steviol glycosides (WHO, 2006). These estimates are presented in Table 3. Merisant also listed intended use levels of rebaudioside A for various food applications in their GRAS Notification (Table 4). Merisant utilized food consumption survey data from 2003-2004 NHANES to determine the estimated daily intake from the proposed uses of rebaudioside A. On a per user basis, the mean and 90th precentile daily consumption of rebaudioside A were estimated as 2.0 and 4.7 mg/kg bw/day, respectively. In its notification, Cargill (2008) utilized a different approach in estimating dietary intake figures for rebaudioside A when incorporated as a general sweetener in a broad cross-section of processed foods. Cargill considered that with a few minor exceptions rebaudioside A food uses and use levels would be comparable to the aspartame uses in the US. Using post-market surveillance consumption data and published data for consumption of aspartame and other high intensity sweeteners (Renwick, 1990), Cargill performed a side-by-side consumption analysis for rebaudioside A versus aspartame. Findings from the above-described different sources, along with FSANZ estimates, are further discussed in Section IV.C, and the intake estimates are presented in Table 5.

C. Estimated Daily Intake

The very conservative consumer intake estimates provided by JECFA as shown in Table 3 were utilized to gauge the potential human exposures of steviol glycosides and rebaudioside A in foods as reported in the US and in other countries. Since rebaudioside A is about twice as sweet as the mixed glycosides, these levels can be adjusted accordingly. Compound Solutions intends to use rebaudioside A in a number of food categories at levels that comply with GMP uses. The application of rebaudioside A to the same foods and at the same levels as those described in earlier FDA notices as discussed in Section II.C is unlikely to affect the dietary intake of

[8] Non-nutritive sweeteners: Substances having less than 2 percent of the caloric value of sucrose per equivalent unit of sweetening capacity.
[9] See 21 CFR 182.1(b)(1).

rebaudioside A from introduction into the market by another supplier who will have to compete in essentially the same markets and foods. This also negates the need for a cumulative intake analysis.

Table 3. Food Uses of Steviol Glycosides Reported to JECFA with Calculated Steviol Equivalents

Food Type	Maximum Use Level Reported[a] (mg steviol glycosides /kg of food)	Maximum Use Level Calculated for Rebaudioside A[b] mg rebaudioside A /kg of food	Maximum Use Level Calculated for Rebaudioside A[b] mg Steviol Equivalents /kg of food
Desserts	500	250	83
Cold confectionery	500	250	83
Pickles	1000	500	167
Sweet corn	200	100	33
Biscuits	300	150	50
Beverages	500	250	83
Yogurt	500	250	83
Sauces	1000	500	167
Delicacies	1000	500	167
Bread	160	80	27

[a] Reproduced from WHO, 2006.
[b] Calculated by Expert Panel assuming twice the sweetness intensity for rebaudioside A and three-fold difference in molecular weight between rebaudioside A and steviol.

Table 4. Proposed Uses & Levels of Rebaudioside A by Merisant[a]

Food Group	Rebaudioside A (ppm)
Tabletop sweeteners	30,000[b]
Sweetened ready-to-drink teas	90-450
Fruit juice drinks	150-500
Diet soft drinks	150-500
Energy drinks	150
Flavored water	150
Cereals (oatmeal, cold cereal, cereal bars)	150

[a] Merisant, 2008.
[b] Reb A content of sachet prior to dilution and not representative of "as consumed."

Table 5. Summary of Estimated Daily Intake Assessments for Rebaudioside A & Calculation of Rebaudioside A Values from JECFA & FSANZ Estimates of the EDI

Scenarios	EDI		
	As Steviol[a] (mg/kg bw/day)	As Rebaudioside A[b] (mg/kg bw/day)	Total Daily Intake[c] (mg/day)
JECFA			
100% Reb A replacement of sugars	5.0	7.5	450
20-30% Reb A replacement of sugars	1.0 - 1.5	1.5 - 2.3	90 - 140
FSANZ			
100% Reb A replacement of sugars	0.3 - 1.0	0.5 - 1.5	30 - 90
MERISANT			
		2.0 - 4.7[d]	120 - 282
CARGILL			
		1.3 - 3.4[d]	78 - 204

[a] Published values for mixed steviol glycosides consumption listed in this column were used for the calculation of Reb A consumption values appearing in next two columns.
[b] Estimates for Reb A consumption were calculated from JECFA and FSANZ estimates as steviol by multiplying by 3 to correct for the molecular weight of Reb A compared to steviol and by subsequently dividing by 2 because of the increased inherent sweetness of Reb A compared to the mixed steviol glycosides.
[c] Total daily intake figures were calculated for a 60 kg adult.
[d] Published values are shown for comparison purposes.

Further consideration was given to anticipated human exposures as projected independently and with different approaches by JECFA (WHO, 2006), Merisant (2008), and Cargill (2008). As described below, the multiple approaches tended to converge to yield estimated daily intakes (EDIs) in the range of 1.3 – 4.7 mg/kg bw/day that, when compared to the acceptable daily intake (ADI), constitutes an integral component in the subject GRAS evaluation.

JECFA evaluated information on exposure to steviol glycosides as submitted by Japan and China. Additional information was available from a report on *Stevia rebaudiana* Bertoni plants and leaves that were prepared for the European Commission by the Scientific Committee on Food. JECFA used the GEMS/Food database to prepare international estimates of exposure to steviol glycosides (as steviol). JECFA assumed that steviol glycosides would replace all dietary sugars, at the lowest reported relative sweetness ratio for steviol glycosides and sucrose, which is 200:1. The intakes ranged from 1.3 mg/kg bw/day with the African diet to 3.5 mg/kg bw/day with the European diet. Additionally, JECFA also estimated the per capita exposure derived from disappearance (poundage) data supplied by Japan and China. The

Committee evaluated exposures to steviol glycosides by assuming full replacement of all dietary sugars in the diets for Japan and the US. The exposures to steviol glycosides (as steviol) as evaluated or derived by the Committee are summarized in Table 6.

Table 6. Summary of Estimates of Exposure to Steviol Glycosides (as Steviol)

ESTIMATE	EXPOSURE (mg/kg BW/DAY)
GEMS/Food (International)[a]	1.3--3.5 (for a 60 kg person)
Japan, Per Capita	0.04
Japan, Replacement Estimate[b]	3
US, Replacement Estimate[b]	5

[a] WHO Global Environment Monitoring System — Food Contamination Monitoring and Assessment Programme.
[b] These estimates were prepared in parallel to those for the international estimates; it was assumed that all dietary sugars in diets in Japan and the US would be replaced by steviol glycosides on a sweetness equivalent basis, at a ratio of 200:1.

In its assessment, JECFA concluded that the replacement estimates were highly conservative as the calculated dietary exposure overestimates likely consumption and that true dietary intakes of steviol glycosides (as steviol) would probably be 20 – 30% of these values or 1.0 - 1.5 mg/kg bw/day on a steviol basis, or 3.0 – 4.5 mg/kg bw/day for rebaudioside A based on the molecular weight adjustment. Furthermore, by adjusting for the 400-fold increased sweetness of rebaudioside A relative to sucrose compared to the mixed steviol glycosides sweetness factor of 200-fold relative to sucrose assumed by JECFA, the estimated dietary intake of rebaudioside A would likely be about 1.5 to ~ 2.3 mg/kg bw/day.

Similar to JECFA, FSANZ (2008) also estimated steviol glycoside dietary intake for adult consumers in New Zealand, assuming a full sugar replacement scenario which resulted in estimated exposures of 0.3 - 1.0 mg/kg bw/day on a steviol basis, or 0.5 – 1.5 mg/kg bw/day for rebaudioside A when making both the molecular weight and sweetness equivalency calculations. Merisant also calculated a dietary estimate for rebaudioside A of 2.0 mg/kg bw/day for the average consumer of the foods listed in Table 4 and 4.7 mg/kg bw/day for a 90th percentile consumer. In another review conducted on behalf of Cargill and included in their GRAS notification, the intake of rebaudioside A when used as a complete sugar replacement was estimated at 1.3 – 3.4 mg/kg bw/day when calculated as rebaudioside A (Renwick, 2008). The estimated daily intake assessments have been compiled in Table 5. These different assessments suggest that total daily consumption of rebaudioside A for specified food categories and as a general purpose sweetener is unlikely to exceed 5 mg/kg bw/day, for a total daily dietary exposure of up to 300 mg rebaudioside A for an adult weighing 60 kg.

In October 2009, Cargill applied to FSANZ to increase the maximum usage levels of high purity steviol glycosides in the high volume food categories of ice cream and various beverages. Cargill supported its application with increased usage levels by presenting market share analyses which overestimate actual intake while remaining well below the generally accepted ADI. In December

2010, FSANZ recommended accepting the increased usage levels as requested since no public health and safety issues were identified. Final action is expected to materialize in 2011 (FSANZ, 2010).

On January 13, 2011, EFSA revised its dietary exposure assessment of steviol glycosides. For high consumers, revised exposure estimates to steviol glycosides remain above the established acceptable daily intake (ADI) of 4 mg/kg bw (steviol equivalent). For European children aged 1-14, revised intake estimates ranged from 1.7 to 16.3 mg/kg bw/day, and for adults, the range was reported to be from 5.6 to 6.8 mg/kg bw/day (EFSA, 2011).

There have been many scholarly estimates of potential dietary intake of replacement sweeteners--- including steviol glycosides---that have been published (FSANZ, 2008; Renwick, 2008; WHO, 2003) or submitted to FDA (Merisant, 2008). In a recent GRAS notification 301, a simplified estimate was proposed to and accepted by FDA, based on the estimates of exposure in "sucrose equivalents" (Renwick, 2008) and the sweetness intensity of any particular sweetener (BioVittoria, 2009). As summarized in GRN 301, the 90th percentile consumer of a sweetener which is 100 times as sweet as sucrose when used as a total sugar replacement would be a maximum of 9.9 mg/kg bw/day for any population subgroup. As noted in Table 2, the minimum sweetness intensity for Compound Solutions' steviol glycosides preparation is 300-fold that of sucrose. Therefore, the 90th percentile consumer of steviol glycosides preparations would consume no more than one-third this level or less than 3.3 mg/kg bw/day. Based on an estimate that steviol glycosides preparations consist of 40% steviol equivalents,[10] the consumption of steviol glycosides would be less than 2 mg/kg bw/day (approximately 1.3 mg/kg bw/day) on a steviol equivalence basis.

The extent that stevia-based sweeteners will penetrate the US food supply and the extent the market will select mixed steviol glycoside products versus Reb A products remains uncertain. Furthermore, many competing non-caloric sweeteners are currently available to consumers, which have been successful in the marketplace, most notably aspartame and sucralose.

Based on the totality of dietary intake considerations presented above, the intake estimates are viewed as being conservative. When comparing these EDI assessments for steviol glycosides, we see that total daily consumption of the steviol glycosides and Reb A for defined food uses and as a general purpose sweetener is expected to be substantially less than the acceptable daily intake values discussed at length in Section VI.B.

D. Other Information on Human Exposure to Stevia: Use as Food Ingredient & Other Uses

For about 20 years, consumers in Japan and Brazil, where stevia has long been approved as a food additive, have been using stevia extracts as non-caloric sweeteners.[11] It was reported that 40% of the artificial sweetener market in Japan is stevia based and that stevia is commonly used in processed foods in Japan (Lester, 1999). Although there are no reported uses of rebaudioside

[10] Calculated by Expert Panel by multiplying by the ratio of molecular weight of steviol to molecular weight of stevioside.

[11] See Raintree NutritionTropical Plant Database. http://www.rain-tree.com/stevia.htm

A as a dietary supplement, use of steviol glycoside as a dietary supplement is presently permitted in the US, Canada, Australia, and New Zealand and as a natural health product in Canada. It has wide use in China and Japan in food and in dietary supplements. In 2005, it was estimated that sales of stevia in the US reached $45 million (The Food Institute Report, 2006). More recent reports of consumption figures for stevia reveal pronounced increases in global consumption. Worldwide, Zenith International estimates stevia sales of 3500 metric tons in 2010 which represents a 27% increase over 2009 figures. The market value is estimated to have increased to $285 million (Zenith, 2011).

Hawke (2003) reported that stevia is commonly used as a treatment for Type 2 diabetes in South America. However, for its therapeutic effects elevated doses in the range of 1 g/person/day or more were reported to be necessary (Gregersen et al., 2004).

V. SAFETY DATA FOR REBAUDIOSIDE A

A. Safety Data on Steviol Glycosides: Recent Reports & Reviews by Expert Bodies & Other Scientists

Stevia and steviol glycosides have been extensively investigated for their biological, toxicological, and clinical effects (Carakostas et al., 2008; Geuns, 2003; Huxtable, 2002). Additionally, the national and international regulatory agencies have thoroughly reviewed the safety of stevia and its glycosides. Most notably, over the years JECFA has evaluated stevia and steviol glycoside multiple times (WHO, 2000, 2006, 2007, 2008). Recently FSANZ (2008) also evaluated steviol glycosides for use in food. The JECFA reviews, as well as the other reviews completed before 2008, primarily focused on mixtures of steviol glycosides typically and were not specific for purified rebaudioside A.

From the safety perspective, some of the earliest studies on steviol glycosides were of limited value as the actual compositions of materials investigated and their questionable purities undermined drawing firm toxicological conclusions. These early studies reported a decrease in fertility with crude stevia preparations and increased mutagenic activity of the principle metabolite, steviol. Based on these and other questions raised about safety by studies with materials of lesser purity and by studies with unusual protocols in *in vivo* and in *in vitro* systems usually employing high doses or high concentrations of test materials, FDA was reluctant to authorize the use of stevia. These concerns included renal toxicity, effects on glucose metabolism, and inhibition of mitochondrial enzymes. Over the last decade and half, the safety of steviol glycosides and rebaudioside A in particular have been extensively investigated employing comprehensive and modern toxicology protocols using scientifically accepted dosing regimens of purified and standardized test substances. The findings from these investigations are discussed below. [12]

JECFA encouraged the further elucidation of clinical effects on blood pressure and glucose metabolism on hypertensive and diabetic individuals, respectively, in parallel with normal human subjects. By 2006, sufficient data were generated for JECFA to satisfactorily establish a temporary ADI, which was finalized in 2008. Additional details on the JECFA reviews are discussed below.

1. Summary of JECFA Reviews

Earlier at its 51st meeting, JECFA (WHO, 2000) expressed the following reservations about the safety data available at that time for steviol glycosides:

[12] Recently, an additional subchronic study was published that investigated the effects of 97% pure stevioside in drinking water on body weight, organ relative weight, hematological and biochemical parameters, and enzyme activities in Sprague Dawley rats. This study is summarized in Appendix E and is discussed by the Expert Panel in Section VI.B.

> The Committee noted several shortcomings in the information available on stevioside. In some studies, the material tested (stevioside or steviol) was poorly specified or of variable quality, and no information was available on other constituents or contaminants. Furthermore, no studies of human metabolism of stevioside and steviol were available. In addition, data on long-term toxicity and carcinogenicity were available for stevioside in only one species. The mutagenic potential of steviol has been tested sufficiently only *in vitro*.

Subsequently, additional data were generated on the metabolism of steviol glycosides and submitted to JECFA. This information suggested that the common steviol glycosides are converted to steviol by intestinal bacteria and then rapidly converted to glucuronides that are excreted. The committee now had a molecular basis to become comfortable with studies on test materials which consisted of variable composition but were relatively high purity mixtures of the common steviol glycosides. The new information also revealed that in *in vitro* studies steviol is mutagenic, while *in vivo* condition it is not mutagenic. The committee became convinced that purified steviol glycosides did not impair reproductive performance as did crude preparations of stevia and that there was sufficient chronic studies in rats with adequate no observed effect levels (NOEL) that could support a reasonable acceptable daily intake (ADI) in the range of doses that would be encountered by the use of steviol glycosides as a sugar substitute. However, JECFA wanted more clinical data to rule out pharmacological effects at the expected doses. The following excerpt was taken from the report of the 63rd meeting (WHO, 2006):

> The Committee noted that most of the data requested at its fifty-first meeting, e.g., data on the metabolism of stevioside in humans, and on the activity of steviol in suitable studies of genotoxicity *in vivo*, had been made available. The Committee concluded that stevioside and rebaudioside A are not genotoxic *in vitro* or *in vivo* and that the genotoxicity of steviol and some of its oxidative derivatives *in vitro* is not expressed *in vivo*.
>
> The NOEL for stevioside was 970 mg/kg bw/day in a long-term study (Toyoda et al., 1997) evaluated by the Committee at its fifty-first meeting. The Committee noted that stevioside has shown some evidence of pharmacological effects in patients with hypertension or with type-2 diabetes at doses corresponding to about 12.5–25 mg/kg bw/day (equivalent to 5–10 mg/kg bw/day expressed as steviol). The evidence available at present was inadequate to assess whether these pharmacological effects would also occur at lower levels of dietary exposure, which could lead to adverse effects in some individuals (e.g., those with hypotension or diabetes).
>
> The Committee therefore decided to allocate a temporary ADI, pending submission of further data on the pharmacological effects of steviol glycosides in humans. A temporary ADI of 0–2 mg/kg bw was established for steviol glycosides, expressed as steviol, on the basis of the NOEL for stevioside of 970 mg/kg bw/day (or 383 mg/kg bw/day, expressed as steviol) in the 2-year study in rats and a safety factor of 200. This safety factor incorporates a factor of 100 for inter- and intra-species differences and an additional factor of 2 because of the need for further information. The Committee noted that this temporary ADI only applies to products complying with the specifications.
>
> The Committee required additional information, to be provided by 2007, on the pharmacological effects of steviol glycosides in humans. These studies should involve repeated exposure to dietary and therapeutic doses, in normotensive and hypotensive individuals and in insulin-dependent and insulin-independent diabetics.

In 2007, at its 68th meeting, JECFA (WHO, 2007) concluded that sufficient progress had been made on the clinical studies and extended the temporary ADI until 2008. Subsequently, sufficient data had been received by JECFA to revise and finalize food additive specifications for steviol

glycosides (FAO, 2007a). The Chemical and Technical Assessment report written after the 2007 meeting, explained the Committee's thinking which resulted in flexibility in the identity specifications (FAO, 2007b).

> In response to the call for data on "stevioside" for the 63rd meeting of the Committee, submissions from several countries showed that the main components of the commercially available extracts of stevia are stevioside and rebaudioside A, in various amounts ranging from about 10-70% stevioside and 20-70% rebaudioside A. The information indicated that most commercial products contained more than 90% steviol glycosides with the two main steviol glycosides comprising about 80% of the material. The 63rd JECFA required that the summed content of stevioside and rebaudioside A was not less than 70% and established a minimum purity of 95% total steviol glycosides. Analytical data showed that most of the remaining 5% could be accounted for by saccharides other than those associated with the individual steviol glycosides.
>
> Noting that the additive could be produced with high purity (at least 95%) and that all the steviol glycosides hydrolyze upon ingestion to steviol, on which the temporary ADI is based, the 68th JECFA decided it was unnecessary to maintain a limit for the sum of stevioside and rebaudioside content. The Committee recognized that the newly revised specifications would cover a range of compositions that could include, on the dried basis, product that was at least 95% stevioside or at least 95% rebaudioside A.

In 2008, based on additional clinical studies, at its 69th meeting, JECFA finalized the evaluation of steviol glycosides (WHO, 2008) and raised the ADI to 0 – 4 mg/kg bw/day and removed the "temporary" designation. The summary of the Committee's key conclusions in the final toxicology monograph addendum (WHO, 2009) were stated as follows:

> From a long-term study with stevioside, which had already been discussed by the Committee at its fifty-first meeting, a NOEL of 970 mg/kg bw per day was identified. At its sixty-third meeting, the Committee set a temporary ADI of 0–2 mg/kg bw for steviol glycosides, expressed as steviol, on the basis of this NOEL for stevioside of 970 mg/kg bw per day (383 mg/kg bw per day expressed as steviol) and a safety factor of 200, pending further information. The further information was required because the Committee had noted that stevioside had shown some evidence of pharmacological effects in patients with hypertension or with type 2 diabetes at doses corresponding to about 12.5–25.0 mg/kg bw per day (5–10 mg/kg bw per day expressed as steviol).
>
> The results of the new studies presented to the Committee at its present meeting have shown no adverse effects of steviol glycosides when taken at doses of about 4 mg/kg bw per day, expressed as steviol, for up to 16 weeks by individuals with type 2 diabetes mellitus and individuals with normal or low-normal blood pressure for 4 weeks. The Committee concluded that the new data were sufficient to allow the additional safety factor of 2 and the temporary designation to be removed and established an ADI for steviol glycosides of 0–4 mg/kg bw expressed as steviol.
>
> The Committee noted that some estimates of high-percentile dietary exposure to steviol glycosides exceeded the ADI, particularly when assuming complete replacement of caloric sweeteners with steviol glycosides, but recognized that these estimates were highly conservative and that actual intakes were likely to be within the ADI range.

2. Summary of FSANZ Review of Steviol Glycosides

In 2008, FSANZ completed a review of the safety of steviol glycosides for use as a sweetener in foods. FSANZ concluded that steviol glycosides are well tolerated and unlikely to have adverse effects on blood pressure, blood glucose or other parameters in normal, hypotensive or diabetic

subjects at doses up to 11 mg/kg bw/day. The FSANZ review discussed the adequacy of the existing database and several new studies, including the clinical studies reviewed by JECFA in the summer of 2007, most notably the work of Barriocanal et al., which was later published in 2008.

In their draft document, FSANZ also indicated that the new data in humans provides a basis for revising the uncertainty factors that were used by JECFA to derive the temporary ADI for steviol glycosides in 2005. In particular, the evidence surrounding the pharmacological effects of steviol glycosides on blood pressure and blood glucose has been strengthened so that the additional 2-fold safety factor for uncertainty related to effects in normotensive or diabetic individuals is no longer required. Therefore, FSANZ established an ADI of 4 mg/kg bw/day for steviol glycosides as steviol equivalents, derived by applying a 100-fold safety factor to the NOEL of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) in a 2-year rat study (FSANZ, 2008).

3. Summary of EFSA Review of Steviol Glycosides

On March 10, 2010, EFSA adopted a scientific opinion on the safety of steviol glycosides (mixtures that comprise not less than 95% of stevioside and/or rebaudioside A) as a food additive. Earlier---in 1984, 1989 and 1999---the Scientific Committee for Food (SCF) evaluated stevioside as a sweetener. At the time, the SCF concluded that the use of stevioside was "toxicologically not acceptable" due to insufficient available data to assess its safety. However, in light of JECFA's 2008 findings and in response to a June 2008 request by the European Commission, EFSA reevaluated the safety of steviol glycosides as a sweetener. As both rebaudioside A and stevioside are metabolized and excreted by similar pathways, with steviol being the common metabolite for both glycosides, the EFSA Panel agreed that the results of toxicology studies on either stevioside or rebaudioside A are applicable for the safety assessment of steviol glycosides. Considering the available safety data (*in vitro* and *in vivo* animal studies and some human tolerance studies), the EFSA Panel concluded that steviol glycosides, complying with JECFA specifications, are not carcinogenic, genotoxic, or associated with any reproductive/developmental toxicity. The EFSA Panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg bw/day based on the application of a 100-fold uncertainty factor to the NOAEL in the 2-year carcinogenicity study in the rat when administering 2.5% stevioside in the diet. This is equal to 967 mg stevioside/kg bw/day (corresponding to approximately 388 mg steviol equivalents/kg bw/day). Conservative estimates of steviol glycosides exposures both in adults and in children suggest that the ADI could possibly be exceeded by European consumers of certain ages and geographies at the maximum proposed use levels.

Recently, EFSA (2011) revised its exposure assessment of steviol glycosides from its uses as a food additive for children and adults and published the reduced usage levels in 16 foods by a factor of 1.5 to 3, with no changes reported for 12 food groups. Additionally, 15 other foods were removed, mainly within desserts and other products, while 3 new food uses were added. The mean estimated exposure to steviol glycosides in European children (aged 1-14 years) ranged from 0.4 to 6.4 mg/kg bw/day and from 1.7 to 16.3 mg/kg bw/day at the 95th percentile. A correction was considered to be necessary for the consumption of non-alcoholic flavored drinks (soft drinks) by children, and the corrected exposure

estimate at the 95th percentile for children ranged from 1.0 to 12.7 mg/kg bw/day. For adults, the mean and 97.5th percentile intakes were estimated to range from 1.9 to 2.3 and 5.6 to 6.8 mg/kg bw/day, respectively. Non-alcoholic flavored drinks (soft drinks) are the main contributors to the total anticipated exposure to steviol glycosides for both consumer categories. For high consumers, EFSA noted that revised exposure estimates to steviol glycosides remain above the established ADI of 4 mg/kg bw (steviol equivalent).

B. Safety Data on Rebaudioside A

Since 2008, several well-designed toxicology studies that followed the current regulatory and other guidelines for such studies have been reported on purified rebaudioside A. These investigations included additional subchronic studies in rats and one in dogs, mutagenicity studies, reproduction and developmental studies in rats, and comparative pharmacokinetic studies with stevioside in rats and humans, as well as additional clinical studies.

1. Subchronic Studies

Recently, Curry and Roberts (2008) reported the results of two repeat dose studies of rebaudioside A in Wistar rats. The results of these investigations suggest that administration of rebaudioside A to Han Wistar rats at dietary concentrations of up to 100,000 ppm (9938 and 11,728 mg/kg bw/day for males and females, respectively) for 4 weeks or 50,000 ppm (4161 and 4645 mg/kg bw/day for males and females, respectively) for 13 weeks did not present any evidence of systemic toxicity. In the 4-week study, rebaudioside A (97% purity) was administered at dietary concentrations of 0, 25,000, 50,000, 75,000 and 100,000 ppm to male and female rats. The NOAEL, including an evaluation of testes histopathology, was determined to be 100,000 ppm. In the 13-week study, Wistar rats were fed diets containing rebaudioside A at dietary concentrations of 0, 12,500, 25,000 and 50,000 ppm. In high-dose male and females groups, reductions in body weight gain attributable to initial taste aversion and lower caloric density of the feed were observed. Inconsistent reductions in serum bile acids and cholesterol were attributed to physiological changes in bile acid metabolism due to excretion of high levels of rebaudioside A *via* the liver. All other hepatic function test results and liver histopathology were within normal limits. No significant changes in other clinical pathology results, organ weights and functional observational battery test results were noted. Macroscopic and microscopic examinations of all organs were unremarkable with respect to treatment-related findings. The NOAEL in the 13-week toxicity study was considered to be 50,000 ppm or approximately 4161 and 4645 mg/kg bw/day in male and female rats, respectively (Curry and Roberts, 2008).

In another 90-day dietary admix toxicity study, effects of rebaudioside A (99.5% purity) at target exposure levels of 500, 1000 and 2000 mg/kg bw/day were tested in Crl:CD(SD) rats (Nikiforov and Eapen, 2008; Eapen, 2007). Each group consisted of 20/animals/sex. No treatment related effects on clinical observations, food consumption, and functional observational or locomotor activity parameters were noted. There were no treatment related macroscopic, organ weight or microscopic findings. Significantly lower body weight gains were noted in the 2000 mg/kg bw/day group in males but not females. At the end of the dosing period, the body weight in males was 9.1% lower than the control group. Due to the small magnitude of difference from the control

group value, the investigators did not consider this result to be adverse. The decrease was most likely due to the large proportion of the diet represented by the test material. The NOAEL was determined as ≥2000 mg/kg bw/day.

A 6-month dietary toxicity study in Beagle dogs (4/sex/group) was conducted to investigate the potential adverse effects of rebaudioside A (97.5% purity) at dosage levels of 0, 500, 1000 or 2000 mg/kg bw/day (Eapen, 2008). There were no unscheduled deaths during the course of the study. No treatment-related clinical observations were noted. Administration of rebaudioside A did not affect home cage, open field observations and functional observations and measurements. No differences in hematology findings, serum chemistry findings, or urinalysis findings between the groups were noted. Additionally, no treatment related gross necropsy observations, alterations in final body weight, alterations in organ weights, or histological changes were noted. The investigators concluded that no systemic toxicity of rebaudioside A was observed at dosage levels up to 2000 mg/kg bw/day and the assigned NOAEL was ≥2000 mg/kg bw/day.

2. Mutagenicity Studies

In a set of *in vitro* and *in vivo* genotoxicity assays covering mutation, chromosome damage and DNA strand breakage, rebaudioside A consistently and uniformly revealed negative results (Pezzuto et al, 1985; Nakajima, 2000a, b; Sekihashi et al., 2002. These studies were critically reviewed by Brusick (2008). JECFA also reviewed an unpublished chromosome aberration assay of rebaudioside A in cultured mammalian cells (Nakajima, 2000a) and did not find increases in chromosome aberrations.

Additionally, FDA also reviewed three unpublished studies on rebaudioside A including a bacterial mutagenicity study (Wagner and Van Dyke, 2006), a mouse lymphoma study (Clarke, 2006) and a mouse micronucleus study (Krsmanovic and Huston, 2006) submitted by Merisant as part of the GRAS Notification. All three studies demonstrated lack of mutagenic or genotoxic activity. Additionally, Williams and Burdock (2009) also reported lack of genotoxicity in another set of published studies that included *in vitro* mutagenicity assays with *Salmonella, E. coli,* and mouse lymphoma cells. These investigators also reported lack of *in vitro* clastogenic effects in Chinese hamster V79 cells and the absence of *in vivo* effects in a mouse micronucleus assay and a rat study for unscheduled DNA synthesis. The key mutagenicity testing results for rebaudioside A are summarized in Table 7.

3. Reproduction & Developmental Studies

In a two-generation reproductive toxicity study, rebaudioside A (97 % purity) at 0, 7,500, 12,500, and 25,000 ppm was administered in diet to male and female Han Wistar rats (Curry, et al., 2008). Administration of rebaudioside A was not associated with any signs of clinical toxicity or adverse effects on body weight, body weight gain, or food consumption. Similarly, administration of rebaudioside A did not affect reproductive performance parameters including mating performance, fertility, gestation lengths, estrous cycles, or sperm motility, concentration, or morphology in either the F_0 or F_1 generations. The survival and general condition of the F_1 and F_2 offspring, their pre-weaning reflex development, overall body weight gains, and the timing of

sexual maturation, were not adversely affected by rebaudioside A treatment. The NOAEL for reproductive effects was 25,000 ppm and the NOAEL for the survival, development, and general condition of the offspring also was considered to be 25,000 ppm or 2,048 to 2,273 mg/kg body weight/day (the highest dose tested).

The results from two unpublished studies with rebaudioside A (Sloter 2008a, b) further support the above described findings from published studies. In a two-generation dietary reproduction study, four groups of male and female Crl:CD(SD) rats (30/sex/group) were fed either basal diet or the diet containing rebaudioside A (purity 95.7%) for at least 70 consecutive days prior to mating (Sloter 2008a). For the F_0 and F_1 generations rebaudioside A doses were 0, 500, 1000 and 2000 mg/kg/day. At initiation of study, F_0 animals were approximately 7 weeks of age. The test diet was offered to the offspring selected to become the F_1 generation following weaning [beginning on postnatal day (PND) 21]. The F_0 and F_1 males continued to receive rebaudioside A throughout mating, continuing through the day of euthanasia. The F_0 and F_1 females continued to receive rebaudioside A throughout mating, gestation and lactation until day of euthanasia. The authors concluded that there were no effects on reproduction in males or females as evaluated by estrus cycles, mating, fertility, conception or copulation indices, number of days between pairing and coitus, gestation length, and spermatogenic endpoints. Both for parental systemic and reproductive toxicity a dose level ≥2000 mg/kg bw/day (highest dose administered) was assigned to be the NOAEL.

In an embryo/fetal developmental toxicity study in rats (Sloter, 2008b), effects of rebaudioside A administered via gavage was tested. Rebaudioside A administration did not affect intrauterine growth and survival, and there were no test article-related fetal malformations or developmental variations at any dosage level. In the absence of maternal or developmental toxicity a dose level ≥ 2000 mg/kg bw/day (highest dose administered) was considered to be the NOAEL for maternal and embryo/fetal developmental toxicity.

4. Clinical Studies on Rebaudioside A

In a four week randomized, double-blind, placebo controlled trial, hemodynamic effects of rebaudioside A at a dose of 1000 mg/day rebaudioside A (97% purity) or placebo in 100 individuals with normal and low-normal systolic blood pressure (SBP) and diastolic blood pressure (DBP) were investigated (Maki et al., 2008a). Subjects were predominantly female (76%, rebaudioside A and 82%, placebo) with a mean age of ~41 (range 18 to 73) years. At baseline, mean resting, seated SBP/DBP was 110.0/70.3 mm Hg and 110.7/71.2 mm Hg for the rebaudioside A and placebo groups, respectively. Compared with placebo, administration of rebaudioside A did not significantly alter resting, seated SBP, DBP, mean arterial pressure (MAP), heart rate (HR) or 24-hour ambulatory blood pressure responses. The investigators concluded that consumption of 1000 mg/day of rebaudioside A produced no clinically important changes in blood pressure in healthy adults with normal and low-normal blood pressure.
In another trial, effects of 16 weeks of consumption of 1000 mg rebaudioside A (97% purity, n = 60) were compared to placebo (n = 62) in men and women (33-75 years of age) with type 2 diabetes mellitus (Maki, et al., 2008b). Changes in glycosylated hemoglobin levels did not differ significantly between the rebaudioside A (0.11 ± 0.06%, mean ± standard error) and placebo

Table 7. Mutagenicity Studies on Rebaudioside A

End-Point	Test System	Material	Purity (%)	Concentration / Dose	Result	Reference
Bacterial Mutagenicity	5 Salmonella strains with and without exogenous metabolic activation system	Reb A	99.5	1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate	No mutagenic response	Wagner and Van Dyke (2006)
Bacterial Mutagenicity	4 Salmonella strains and 1 *E. coli* strain with and without exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg per plate	No mutagenic response	Williams and Burdock (2009)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	99.5	Cloning conc. of 500, 1000, 2000, 3000, 4000 and 5000 µg/mL	No mutagenic or clastogenic response	Clarke (2006)
Mouse Lymphoma	L5178Y/TK+/- mouse lymphoma mutagenesis assay in the absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Chromosome Aberration	Human lymphocytes in absence and presence of exogenous metabolic activation system	Reb A	95.6	Up to 5000 µg/mL	No mutagenic or clastogenic response	Williams and Burdock (2009)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female ICR mice	Reb A	99.5	500, 1000 and 2000 mg/kg bw	No increase in micronuclei formation	Krsmanovic and Huston (2006)
Mouse Micronucleus	Micronucleus study in groups of 5 male and 5 female NMRI mice	Reb A	95.6	Up to 750 mg/kg bw	No increase in micronuclei formation	Williams and Burdock (2009)
Unscheduled DNA Synthesis	Unscheduled DNA synthesis in one group of 4 Wistar rats	Reb A	95.6	Up to 2000 mg/kg bw	No increase in unscheduled DNA synthesis	Williams and Burdock (2009)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	Stevia extract	Stevio-side, 52%; Reb A, 22%	250 - 2000 mg/kg bw	Negative[a]	Sekihashi et al. (2002)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Reb A	NS	1.2 - 55 mg/mL	Negative[b]	Nakajima (2000a)
Micronucleus formation	BDF1 mouse bone marrow	Reb A	NS	500-2000 mg/kg bw per day for 2 days	Negative[c]	Nakajima (2000b)
Forward mutation	*S. typhimurium* TM677	Reb A	NS	10 mg/plate	Negative[b]	Pezzuto et al. (1985)

NS = Not specified.
[a] Sacrificed at 3 hours and 24 hours.
[b] With or without metabolic activation (source not specified in original monograph).
[c] Sacrificed at 30 hours after 2nd administration.

(0.09 ± 0.05%; p = 0.355) groups. Similarly, no significant (p > 0.05 for all) changes from baseline for rebaudioside A and placebo, respectively, in fasting glucose (7.5 ± 3.7 mg/dL and 11.2 ± 4.5 mg/dL), insulin (1.0 ± 0.64 µU/mL and 3.3 ± 1.5 µU/mL), and Cpeptide (0.13 ± 0.09 ng/mL and 0.42 ± 0.14 ng/mL) were noted. No treatment related changes in blood pressure, body weight, and fasting lipids were noted. Rebaudioside A was well-tolerated, and records of

hypoglycemic episodes showed no excess versus placebo. Based on these results, the investigators suggested that chronic use of 1000 mg rebaudioside A does not alter glucose homeostasis or blood pressure in individuals with type 2 diabetes mellitus.

5. Absorption, Distribution, Metabolism & Excretion (ADME) Studies

In three recently completed studies, absorption and fate of rebaudioside A was systematically investigated in rats and humans.

For comparative purposes to determine whether toxicological studies conducted previously with stevioside would be applicable to the structurally-related glycoside, rebaudioside A, toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol were examined in rats (Roberts and Renwick, 2008). Orally administered single doses of the radiolabeled compounds were extensively and rapidly absorbed with plasma concentration-time profiles following similar patterns for stevioside and rebaudioside A. Within 72 hours of administration, elimination of radioactivity from plasma was essentially complete. All plasma samples had similar metabolite profiles; the predominant radioactive component in all samples was steviol, with lower amounts of steviol glucuronide(s) and low levels of one or two other metabolites. Rebaudioside A, stevioside, and steviol were metabolized and excreted rapidly, with the majority of the radioactivity eliminated in the feces within 48 hours. Urinary excretion accounted for less than 2% of the administered dose for all compounds in both intact and bile duct-cannulated rats, and the majority of the absorbed dose was excreted *via* the bile. After administration of the compounds to intact and bile duct-cannulated rats, radioactivity in the feces was present primarily as steviol. The predominant radioactive compound detected in the bile of all cannulated rats was steviol glucuronide(s), indicating de-conjugation in the lower intestine. The authors concluded that the overall data on toxicokinetics and metabolism indicate that rebaudioside A and stevioside are handled in an almost identical manner in the rat after oral dosing.

In a randomized, double blind, cross-over study in healthy male subjects, Wheeler et al. (2008) assessed the comparative pharmacokinetics of steviol and steviol glucuronide following single oral doses of rebaudioside A and stevioside. Following administration of rebaudioside A or stevioside, steviol glucuronide appeared in the plasma of all subjects, with median Tmax values of 12.00 and 8.00 hours post-dose, respectively. Steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for both compounds. Administration of rebaudioside A resulted in a significantly (approximately 22%) lower steviol glucuronide geometric mean C_{max} value (1472 ng/ml) than administration of stevioside (1886 ng/mL). The geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A (30788 ng*hr/mL) was approximately 10% lower than after administration of stevioside (34090 ng*hr/mL). Steviol glucuronide was excreted primarily in the urine of the subjects during the 72-hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively. No steviol glucuronide was detected in feces. Pharmacokinetic analysis indicated that both rebaudioside A and stevioside were hydrolyzed to steviol in the gastrointestinal tract prior to absorption. The majority of circulatory steviol was in the form of steviol glucuronide indicating rapid first-pass conjugation prior to urinary excretion. Only a small amount of steviol was detected in urine (rebaudioside A: 0.04%; stevioside: 0.02%). The investigators concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in

humans with steviol glucuronide excreted primarily in the urine and steviol in the feces. No safety concerns were noted as determined by reporting of adverse events, laboratory assessments of safety or vital signs.

Another pharmacokinetic investigation was done as a toxicokinetic (TK) phase of a dietary study to determine the potential of rebaudioside A toxicity in rats at levels up to 2000 mg/kg bw/day (Sloter, 2008a). Rebaudioside A and total steviol were detected in peripheral blood of rats during daily administration of 2000 mg/kg bw/day of rebaudioside A at extremely low levels, with mean plasma concentrations of approximately 0.6 and 12 ug/mL, respectively. Estimates of absorbed dose for rebaudioside A and total steviol were approximately 0.02% and 0.06%, respectively, based on the amounts measured in urine collected over 24 hours in comparison to daily administered dietary dose to rats. Mean fecal rebaudioside A and measured hydrolysis products expressed as *Total Rebaudioside A Equivalents* compared to daily administered dose results in an estimate of percent of dose recovered ≈ 84%.

VI. DISCUSSION OF GRAS CRITERIA & REVIEWED INFORMATION

A. GRAS Criteria

FDA defines "safe" or "safety" as it applies to food ingredients as:

> "...reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance."[13]

Amplification is provided in that the determination of safety is to include probable consumption of the substance in question, the cumulative effect of the substance and appropriate safety factors. It is FDA's operational definition of safety that serves as the framework against which this evaluation is provided.

Furthermore, in discussing GRAS criteria, FDA notes that:

> "...General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food."
>
> "General recognition of safety through experience based on common use in food prior to January 1, 1958, shall be based solely on food use of the substance prior to January 1, 1958, and shall ordinarily be based upon generally available data and information."[14]

FDA discusses in more detail what is meant by the requirement of general knowledge and acceptance of pertinent information within the scientific community, i.e., the so-called "common knowledge element," in terms of the two following component elements:[15]

- Data and information relied upon to establish safety must be generally available, and this is most commonly established by utilizing published, peer-reviewed scientific journals; and
- There must be a basis to conclude that there is consensus (but not unanimity) among qualified scientists about the safety of the substance for its intended use, and this is established by relying upon secondary scientific literature such as published review articles, textbooks, or compendia, or by obtaining opinions of expert panels or opinions from authoritative bodies, such as JECFA and the National Academy of Sciences.

---

[13] See 21 CFR 170.3(i).
[14] See 21 CFR 170.30(a).
[15] See 62 FR 18938 (17 April 1997). http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/ucm083058.htm.

The apparent imprecision of the terms "appreciable", "at the time" and "reasonable certainty" demonstrates that the FDA recognizes the impossibility of providing absolute safety, in this or any other area (Lu 1988; Renwick 1990).

As noted below, the safety assessment to ascertain GRAS status for rebaudioside A with the defined food uses meets FDA criteria for reasonable certainty of no harm by considering both the technical and common knowledge elements.

B. Discussion of Expert Safety Reviews of Steviol Glycosides

Because of their sweetness characteristics, steviol glycosides have viable uses as a non-nutritive sweetener in foods.[16] Periodic reviews by JECFA over the years indicate the progress of knowledge on the toxicology of steviol glycosides. Several early safety-related studies on these compounds were performed on crude extracts of stevia. These studies also included multiple investigations with *in vivo* and *in vitro* models which explored the biological activity of stevia extracts at high doses or high concentrations. These early investigations raised several concerns, including impairment of fertility, renal effects, interference with glucose metabolism, and inhibition of mitochondrial enzymes. In recent years as more and more studies were performed on purified glycosides, the toxicology profile of steviol glycosides eventually proved to be rather unremarkable. A number of subchronic, chronic and reproductive studies have been conducted in laboratory animals. These studies were well designed with appropriate dosing regimens and adequate numbers of animals to maximize the probability of detection of important effects. Notably, the initially reported concerns related to the effects of stevia leaves or crude extracts on fertility were refuted by the well-designed reproductive studies with purified steviol glycosides. All other concerns failed to manifest themselves at the doses employed in the long-term rat studies.

The Panel also noted findings from a recently published exploratory subchronic toxicity study in rats by Awney et al. (2010), where series of toxicological effects of stevioside (1500 mg/kg/day) treatment were reported. Critical review of the publication revealed the poor design that included insufficient numbers of animals, group-housing with the potential for stress-related changes, unreliable access to steviol via drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection which affects many chemistry and hematological values, no urine collection and no histopathological evaluations for confirmation of findings beyond the controls. Additionally, the study did not adequately report mean or individual organ weight data, and it lacked comparison of study findings against laboratory historical control

[16] It has also been reported that steviol glycosides may have pharmacological properties, which can be used to treat certain disease conditions such as hypertension and Type 2 diabetes. Chatsudthipong and Muanprasat (2009) published a comprehensive review where they note that such therapeutic applications have not been firmly established as being due to steviol glycosides. The reviewers point out that the effects occur at higher doses than would be used for sweetening purposes. Furthermore, many effects noted in older studies may have been due to impurities in preparations that do not meet the contemporary purity specifications established by JECFA for use as a sweetener. If oral doses of steviol glycosides impart pharmacological effects, such effects would undoubtedly occur due to actions of the principle metabolite, steviol, but the pharmacological effects of steviol have not been comprehensively investigated.

data. Contrary to the data presented in this study, several well-designed studies did not reveal any adverse effects of steviol glycoside.

As discussed in Section V, at its fifty-first meeting, JECFA determined that there were adequate chronic studies in rats, particularly the study by Toyoda et al. (1997), to establish a temporary ADI of 0 - 2 mg/kg bw/day with an adequate margin of safety. The committee also critically reviewed the lack of carcinogenic response in well-conducted studies. These studies justified the Committee conclusion that the *in vitro* mutagenic activity of steviol did not present a risk of carcinogenic effects *in vivo* and, therefore, all common steviol glycosides which share the same basic metabolic and excretory pathway and that the use of high purity preparations of various steviol glycosides are safe to use as a sugar substitute. Subsequently, the additional clinical data reviewed by JECFA allowed the Committee to establish a permanent ADI of 0 - 4 mg/kg bw/day (based on steviol equivalents) or 0 - 12 mg/kg bw for rebaudioside A. The GRAS Expert Panel critically reviewed the JECFA assessment and agrees with this reasoning.

The Panel also noted from a recent study that DNA damage was seen in a variety of organs as assessed by comet assay in rats given drinking water containing 4 mg/mL steviol glycosides for up to 45 days (Nunes et al., 2007). The methodology used in this study was questioned by several experts in the field (Geuns, 2007; Williams, 2007; Brusick, 2008). The Panel has reviewed the cited publications and agrees and discounts the importance of the Nunes et al. (2007) study.

The Panel has reviewed the findings from human clinical studies. The Panel noted that as regards to the clinical effects noted in humans, in order to corroborate the observations in these studies that these effects of steviol glycosides only occur in patients with either elevated blood glucose or blood pressure (or both), JECFA called for studies in individuals that are neither hypertensive nor diabetic (WHO, 2006). The new data presented to JECFA and also published by Barriocanal et al. (2008) demonstrate the lack of pharmacological effects of steviol glycosides at 11 mg/kg bw/day in normal individuals or approximately slightly more than 4 mg/kg bw on the basis of steviol equivalents. It is possible that JECFA may also have reviewed the preliminary results associated with the recently published clinical studies on rebaudioside A (Maki et al., 2008a, b). The Panel concludes that there will be no effects on blood pressure and glucose metabolism in humans at the doses of rebaudioside A expected from its use in food as a non-nutritive sweetener.

JECFA's review also included anticipated dietary patterns and the use concentrations expected in various foods in order to calculate an estimated daily intake (EDI) (WHO, 2003, 2006). Based on the assumption of 100% substitution of steviol glycosides for sugar, an EDI of 5 mg/kg bw/day of steviol was calculated for US consumption. JECFA noted that the replacement estimates were highly conservative and that this calculated intake of steviol glycosides (as steviol) would more likely be 20–30% of these values. Except for the scenario developed by JECFA with 100% replacement of sugars by steviol glycosides, and as discussed in Section IV.C and summarized in Table 5, the highest dietary estimate for use in foods for rebaudioside A is 4.7 mg/kg bw/day. The Panel agrees with the JECFA ADI of 4 mg/kg bw/day based on steviol equivalents which corresponds to 12 mg/kg bw/day for rebaudioside A and notes that the estimates as contained in Table 5 of anticipated dietary intake are below the ADI.

C. Discussion of Rebaudioside A Safety[17]

Since July 2008, over ten papers describing the results of a comprehensive research program by different groups on rebaudioside A have been published. These and some other unpublished studies formed the basis of the two initial GRAS notifications to FDA each by Cargill (GRN 253) and Merisant (GRN 252). Prior to this, a limited number of toxicology studies specifically on rebaudioside A were conducted. Even before these new studies were completed and as noted in the previous section, JECFA concluded that seven common steviol glycosides are safe for use as sweetener preparations when present in any combination as long as the combined purity of 95% or more was established.

Since a majority of the previous pharmacokinetic research was conducted with steviol glycosides, the presumed strategy adopted for the more recent research on rebaudioside A was to conduct a limited number of well-designed and executed toxicology studies on rebaudioside A itself and to demonstrate in rats and in humans that it is handled pharmacokinetically similarly to stevioside. This approach appears to have been undertaken to justify the JECFA-generated ADI without having to conduct a chronic study in rats with rebaudioside A. Additionally, the Merisant group conducted three mutagenicity assays on rebaudioside A that FDA generally considers to be most predictive for carcinogenicity potential. The Cargill group conducted two clinical studies to assure that rebaudioside A does not have potentially problematic pharmacological effects on blood glucose and blood pressure.

In a review article, Carakostas et al. (2008) summarized the most recent research on rebaudioside A. This review summarized the findings of the Cargill research program as follows:

- Steviol glycosides, rebaudioside A, and stevioside are not genotoxic *in vitro*.
- In well-conducted *in vivo* assays, steviol glycosides, rebaudioside A, and stevioside have not been found to be genotoxic.
- A report indicating that stevioside produces DNA breakage *in vivo* appears to be flawed (Nunes, et al., 2007) and was improperly interpreted as a positive response.
- Steviol genotoxicity in mammalian cells is limited to *in vitro* tests that may be affected by excessive concentrations of the compound.
- The primary evidence for steviol genotoxicity is derived from very specific bacterial tests or purified plasmid DNA that lack DNA repair capabilities.
- Stevioside is not a carcinogen or cancer promoter in well-conducted rodent chronic bioassays.
- The pharmacokinetic similarity between rebaudioside A and stevioside justifies the use of the ADI established by JECFA that was determined on studies employing stevioside as the main component as the ADI for rebaudioside A.

[17] Questions about the safety of rebaudioside A were previously raised by Huxtable (2002) and Kobylewski and Eckhert (2008). Their respective concerns, as well as opposing views supporting the safety of designated food uses of rebaudioside A expressed by Expert Panels have been outlined in other GRAS notifications that were submitted to FDA. A more detailed account can be found in GRAS notifications 278, 287, 303, and 304.

- The dietary levels expected from consumption of rebaudioside A as a total replacement of sugar (Renwick, 2008) are less than the ADI and, therefore, there is no safety concern for consumers.

The Panel concurs that the consumption estimates described by both JECFA and Renwick (2008) very conservatively represent a potential high user of rebaudioside A as this non-nutritive sweetener becomes more widely available in food. As part of the present GRAS evaluation, the Panel adopts the JECFA EDI for application to Compound Solutions' high purity rebaudioside A (≥ 95%).

Regarding the available aggregate safety information, the Panel has concluded that JECFA has critically and extensively evaluated the use of steviol glycosides in foods and agrees that, at the present time, the ADI for steviol glycosides of adequate purity as defined by JECFA specifications has been properly determined to be 4 mg/kg bw/person as steviol equivalents, which corresponds to 12 mg/kg bw/day on a molecular weight-adjusted basis for rebaudioside A and on a dry weight basis. The Panel agrees that unwanted pharmacological effects are not likely to occur at this level and that high consumers of rebaudioside A are not likely to exceed this level. Therefore, the Panel adopts the JECFA-derived ADI as a safe exposure for rebaudioside A and that food uses meeting the specifications within the limits determined by this esteemed international body of food safety experts can be considered to be generally recognized as safe (GRAS).

The Panel recognizes that JECFA is composed of dozens of scientists that are internationally known experts on food ingredient safety that have established ADIs for food ingredients over the last 40 years. Both Merisant and Cargill took rather rigorous scientific approaches to demonstrate the safety of rebaudioside A. The studies were equally well conducted. The safety profiles compiled by Merisant and Cargill differ somewhat, yet the results are complementary and are mutually reinforcing of rebaudioside A safety.

The studies conducted by Cargill provided significant insight into the pharmacokinetics of rebaudioside A while demonstrating clinical safety of rebaudioside A regarding lack of effects on blood pressure and glucose metabolism that could result from doses expected from use in food. The Merisant notification augmented genotoxicity data in three systems recognized by FDA as good predictors of carcinogenic potential. Two of these assays were conducted in mouse systems. Additional mutagenicity and genotoxicity studies have been published on rebaudioside A (Williams and Burdock, 2009). Merisant added a subchronic study in dogs and a teratology study in rats. Both Cargill and Merisant relied on the JECFA ADI for steviol glycosides as determined largely by published chronic studies in rats. Both groups justified the use of the ADI on pharmacokinetic arguments showing the similarity of stevioside and rebaudioside A metabolism and excretion.

The Panel agrees with the conclusion of JECFA and the Cargill and Merisant Expert Panels that there are a sufficient number of good quality health and safety studies to support the determination that the intended use of purified preparations of steviol glycosides, including rebaudioside A, when added to food at levels up to full replacement of sugar on a sweetness equivalency basis, meets FDA's definition of safe.

D. Common Knowledge Elements of GRAS Determination

The first common knowledge element for a GRAS determination is that data and information relied upon to establish safety must be generally available; this is most commonly established by utilizing published, peer-reviewed scientific journals. The majority of studies reviewed as part of this safety assessment have been published in the scientific literature as reported in Section V. Most of the literature relied upon by JECFA has also been published, most importantly the chronic rat studies on steviol glycosides. JECFA did make limited use of unpublished studies, and they were summarized in the two JECFA monographs. Moreover, JECFA publicly releases the results of their safety reviews, and their meeting summaries and monographs are readily available on their website. Thus, these studies become generally available to the scientific community. JECFA only reviewed a limited number of studies conducted specifically on rebaudioside A. The collection of supporting data on rebaudioside A has recently been enhanced by a series of studies published during 2008 and cited earlier. The newest clinical studies that address JECFA's concern on unwanted pharmacological effects with steviol glycosides (Barriocanal et al., 2008) and with rebaudioside A (Maki et al., 2008a, b) are also published in the peer-reviewed scientific literature.

The Panel recognizes that the safety of steviol glycoside in human foods has been the subject of interest for many years. In addition to the reported substantial history of consumption of stevia, especially in South America and Asia, many scientific studies have been conducted and published. Some of the earlier studies have raised concerns about the safety, and the Panel has given careful attention to such concerns. The overriding evidence has diminished the Panel's concerns based on better study designs, better execution, or simply updated investigations that better reflect state-of-the art toxicological principles and findings.

The remaining common knowledge element for a GRAS determination is that there must be a basis to conclude that there is consensus among qualified scientists about the safety of the substance with its intended use. The JECFA opinion largely meets the common knowledge test on its own. The Panel is cognizant of the scientific rigor and broad base of scientific expertise that resides with the prestigious JECFA. JECFA is composed of expert scientists from various regulatory agencies around the world, as well as other scientists chosen because of their specific expertise on various classes of food ingredients. In addition, FDA participated in the JECFA deliberations.

The JECFA conclusion has been reviewed and validated by other respected regulatory agencies including FSANZ, the Switzerland Office of Public Health, France's Agence Francais De Securite Sanitaire Des Alimenta, and Hong Kong's Centre for Food Safety (FSANZ, 2008; Switzerland Office of Public Health, 2008; AFSSA, 2009; Hong Kong, 2010). Furthermore, the favorable scientific opinion on the safety of steviol glycosides use as a sweetener in foods as issued by EFSA in 2010 reinforces the safety determinations of many other qualified organizations (EFSA, 2010). In addition, a number of individual well-respected scientists have indicated that steviol glycosides are safe for human consumption at doses in the range of the JECFA ADI (Xili et al., 1992; Toyoda et al., 1997; Geuns, 2003; Williams, 2007).

The common knowledge element has been embellished by the many respected scientists that participated in the Cargill-sponsored new research conducted on rebaudioside A, most notably Brusick and Renwick. An assertion of "general recognition of safety" was made by Carakostas et al. (2008). In summary, there are many diverse groups of scientists from all corners of the globe that together provide strong fulfillment of the consensus requirement. Of particular significance from the perspective of establishing consensus for the safety of high purity reb A are the mid-December 2008 "no questions" determinations by FDA for the GRAS notifications for rebaudioside A as submitted by Merisant and Cargill and the more recent comparable findings by FDA with the additional GRAS notifications cited elsewhere.

While the scientific conclusions are not unanimous regarding the safe human food uses of steviol glycosides, the Panel believes that a wide consensus does exist in the scientific community to support the GRAS conclusion on rebaudioside A as outlined in this evaluation. The broader scientific community has concluded that past concerns expressed by others over the years (Huxtable, 2002) and earlier safety issues noted by FDA have been resolved by newer data on more purified test materials and the rigid specifications for purity published by JECFA for steviol glycosides, including rebaudioside A. Indeed, scientists from FDA are members of JECFA and have not objected to the safety decision on steviol glycosides. There is also a wider consensus that the body of new research on rebaudioside A is sufficient as opposed to the small group of scientists that argue that more studies need to be done before the sweetener is made available in the US.

VII. CONCLUSIONS[18]

Compound Solutions' high purity rebaudioside A (≥ 95%) as expressed on a dry weight basis is Generally Recognized As Safe when consumed as a general purpose non-nutritive sweetener in foods other than meat and poultry products when: (1) it is produced in accordance with FDA Good Manufacturing Practices requirements; (2) it meets or exceeds the JECFA purity specifications for steviol glycosides; and (3) it is consumed within the designated JECFA ADI of 12 mg/kg bw/day on a rebaudioside A basis. In order to remain within the designated ADI, it is important to observe good manufacturing practices principles in that the quantity of a substance added to food shall not exceed the amount reasonably required to accomplish its intended technical effect.

This declaration has been made in accordance with FDA's standard for food ingredient safety, i.e., reasonable certainty of no harm under the intended conditions of use.

(b) (6)

Richard C. Kraska, Ph.D., DABT
Chair

(b) (6)

Robert S. McQuate, Ph.D.

(b) (6)

Madhusudan G. Soni, Ph.D., FACN

July 5, 2011

[18] The detailed educational and professional credentials for the individuals serving on the Expert Panel can be found on the GRAS Associates website at www.gras-associates.com. Drs. Kraska and McQuate worked on GRAS and food additive safety issues within FDA's GRAS Review Branch earlier in their careers and subsequently continued working within this area in the private sector. Dr. Soni's curriculum vitae can be accessed at http://www.soniassociates.net. All three panelists have extensive technical backgrounds in the evaluation of food ingredient safety. All three individuals have previously served on multiple GRAS Expert Panels. Dr. Kraska served as Chair of the Panel.

VIII. REFERENCES

Agence Francais De Securite Sanitaire Des Aliments (AFSSA), 2009. http://www.afssa.fr/Documents/AAAT2009sa0119.pdf; http://www.foodnavigator.com/Legislation/France-approves-high-Reb-A-stevia-sweeteners.

Al-Achi, A. and Greenwood, R., 2000. Stevia: a plant for Sweetness? US Pharmacist 25, 12.

Awney, H.A., Massoud, M.I. El-Maghrabi, S., 2010. Long-term feeding effect of stevioside sweetener on some toxicological parameters of growing male rats. Journal of Applied Toxicology, Online Publication: 19 NOV 2010; DOI: 10.1002/jat.1604

Barriocanal, L.A., Palacios, M., Benitez, G., Benitez, S., Jimenez, J.T., Jimenez, N., Rojas, V., 2008. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type 1 and Type 2 diabetics. Regul. Toxicol. Pharmacol. 51, 37-41.

Brusick, D.J., 2008. A critical review of the genetic toxicity of steviol and steviol glycosides. Food Chem. Toxicol., 46(7)(Suppl.1), S83-S91.

Carakostas, M.C., Curry, L.L., Boileau, A.C., Brusick, D.J., 2008. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. Food Chem. Toxicol., 46(7)(Suppl.1), S1-S10.

Cargill GRAS Notification for Rebaudioside A, 2008. Submitted to the US Food and Drug Administration, Washington, DC and identified as GRAS Notification 253. See FDA website at http://www.cfsan.fda.gov/~rdb/opa-grsn.html.

CCFA (Codex Committee on Food Additives), 2009. Proposals for Additions and Changes to the Priority List of Food Additive Proposed for Evaluation by JECFA (CL 2008/26-FA). Codex Alimentarius Commission E, FAO/WHO/JECFA CX/FA 09/41/11.

Chang, S.S., Cook, J.M., 1983. Stability studies of stevioside and rebaudioside A in carbonated beverages. J. Agric. Food Chem. 31, 409-414.

Chatsudthipong, V., Muanprasat, C., 2009. Stevioside and related compounds: therapeutic benefits beyond sweetness. Pharmacol Therapeutics. Jan: 121:41-54.

Clarke, J.J., 2006. *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay) [with Rebaudioside A]. BioReliance, Rockville, MD. Unpublished Report (Study Number AB21TG.704.BTL).

Clos, J.F., DuBois, G.E., Prakash, I., 2008. Photostability of rebaudioside A and stevioside in beverages. J. Agric. Food Chem., 56, 8507-8513.

Curry, L.L., Roberts, A., 2008. Subchronic toxicity of rebaudioside A. Food Chem. Toxicol., 46(7)(Suppl.1), S11-S20.

Curry, L.L., Roberts, A., Brown, N., 2008. Rebaudioside A: two-generation reproductive toxicity study in rats. Food Chem. Toxicol., 46(7)(Suppl. 1), S21-S30.

Eapen, A.K., 2007. A 90-Day Oral (Dietary) Toxicity Study of Rebaudioside A in Rats. WIL Research Laboratories, LLC. Unpublished Report (Study Number WIL-568002).

Eapen, A.K. 2008. A 6-Month Oral (Dietary) Toxicity Study of Chrysanta® 99-P in Beagle Dogs. WIL2006. Research Laboratories, LLC. Unpublished Report (Study Number WIL-568011).

EFSA (European Food Safety Authority), 2010. Scientific Opinion on the safety of steviol glycosides for the proposed uses as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). EFSA Journal 8 (4), 1537 (pages 1-84).

EFSA (European Food Safety Authority), 2011. Revised exposure assessment for steviol glycosides for the proposed uses as a food additive. EFSA Journal 9 (1), 1972,1-19 pp).

European Commission, 1999a. Opinion on Stevia Rebaudiana Bertoni plants and leaves. Scientific Committee on Food (CS/NF/STEV/3 Final, 17 June 1999).

European Commission, 1999b. Opinion on stevioside as a sweetener. Scientific Committee on Food (CS/ADD/EDUL/167Final, 17 June 1999).

FAO (Food and Agriculture Organization), 2007a. Steviol Glycosides. FAO JECFA Monographs 4.

FAO (Food and Agriculture Organization), 2007b. Chemical and Technical Assessment: Steviol Glycosides. Revised by Paul M. Kuznesof, PhD for the 68th JECFA Meeting.

FAO (Food and Agriculture Organization), 2008. Steviol Glycosides. FAO JECFA Monographs 5.

FAO (Food and Agriculture Organization), 2010. Steviol Glycosides. FAO JECFA Monographs 10. http://www/fao/org/ag/agn/jecfa-additives/specs/monograph10/additive-442-m10.pdf.

FCC (Food Chemical Codex), 2009. Rebaudioside A monograph. In- Revisions to FCC Sixth Edition, Second Supplement, pp. 1438-1442.

FSANZ, (Food Standards Australia New Zealand), 2008. Final Assessment Report, Application A540, Steviol Glycosides As Intense Sweeteners.

FSANZ. (Food Standards Australia New Zealand), 2010. Assessment Report, Application A1037, Steviol Glycosides – Increase in Permitted Use Levels.

Geuns, J.M.C., 2003. Molecules of interest stevioside. Phytochemistry 64, 913-921.

Geuns, J.M.C., 2007. Letter to the Editor: Comments to the paper by Nunes et al. (2007). Analysis of genotoxic potentiality of stevioside by comet assay. Food and Chemical Toxicology 45 (2007) 662-666.

Gregersen, S., Jeppensen, P.B., Holst, J.J.,Hermansen, K., 2004. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism, 53, 73–76.

Hawke, J., 2003. The Bittersweet Story of the Stevia Herb. Nexus Magazine vol.10 no. 2. http://www.nexusmagazine.com/articles/stevia.html.

Hanson, J.R., De Oliveira, B.H., 1993. Stevioside and related sweet diterpenoid glycosides. Nat. Prod. Rep.,10, 301-309.

Health Canada, 2009. Revised Guidelines for the Use of Stevia in Natural Health Products. http://www.hc-sc.gc.ca/dhp-mps/prodnatur/legislation/docs/notice-avis-stevia-eng.php

Hong Kong's Centre for Food Safety, 2010. Legislative council brief. Sweeteners in food (amendment) regulation. May. http://www.cfs.gov.hk/english/programme/programme_rafs/files/2010_amendment_to_sweeteners_in_food_regulation_Legco_brief.pdf.

Huxtable, R.J., 2002. Pharmacology and toxicology of stevioside, rebaudioside A, and steviol. In: Kinghorn, A.D. (Ed.) Stevia: The Genus of Stevia. Taylor and Francis Inc., New York.

Kennelly, E.J., 2002. Sweet and non-sweet constituents of Stevia rebaudiana (Bertoni). In: Kinghorn, A.D. (Ed.), Stevia, The Genus Stevia. Medicinal and Aromatic Plants – Industrial Profiles, Vol. 19. Taylor and Francis Inc., London and New York, pp. 68-85.

Kinghorn, A.D., Soejarto, D.D., 1985. Current status of stevioside as a sweetening agent for human use. In: H. Wagner, H. Hikino, and N.R. Farnsworth (Eds.), Economic and Medicinal Plant Research, Vol.1. Academic Press, New York, pp. 1-52.

Kobylewski, S., Eckhert, C.D., 2008. Toxicology of Rebaudioside A: A Review. University of California at Los Angeles.

Krsmanovic, L. ,Huston, T., 2006. Rebaudioside A: Mammalian Erythrocyte Micronucleus Test. BioReliance, Rockville, MD. Unpublished Report (Study Number AB21TG.123.BTL).

Lester, T., 1999. *Stevia rebaudiana.* The Australian New Crops Newsletter, Issue 11, January 1999. www.newcrops.uq.edu.au/newslett/ncn11161.htm.

Lu, F.C., 1988. Acceptable daily intake: inception, evolution and application. Regul. Toxicol. Pharmacol. 8, 45-60.

Maki, K.C., Curry, L.L., Carakostas, M.C., Tarka, S.M., Reeves, M.S., Farmer, M.V., McKenney, J.M., Toth, P.D., Schwartz, S.L., Lubin, B.C., Dicklin, M.R., Boileau, A.C., Bisognano, J.D., 2008a. The hemodynamic effects of rebaudioside A in healthy adults with normal and low-normal blood pressure. Food Chem. Toxicol., 46(7)(Suppl.1), S47-S53.

Maki, K.C., Curry, L.L., Reeves, M.S., Toth, P.D., McKenney, J.M., Farmer, M.V., Schwartz, S.L., Lubin, B.C., Boileau, A.C., Dicklin, M.R., Carakostas, M.C., Tarka, S.M., 2008b. Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. Food Chem. Toxicol., 46(7)(Suppl. 1), S40-S46.

Merisant GRAS Notification for Rebaudioside A, 2008. Submitted to the US Food and Drug Administration, Washington, DC under the name Whole Earth Sweetener Company LLC and identified as GRAS Notification 252. http://www.accessdata.fda.gov/scripts/fc/fcnNavigation.cfm?rpt=grasListing.

Nakajima, (initials unknown), 2000a. Chromosome aberration assay of rebaudioside A in cultured mammalian cells. Test number 5001 (079–085). Ministry of Health and Welfare, Japan.

Nakajima, (initials unknown), 2000b. Micronucleus test of rebaudioside A in mice. Test number 5002 (079-086). Unpublished report of a study conducted at the Biosafety Research Center, Japan. Submitted to WHO by Ministry of Health and Welfare, Japan.

Nanayakkara, N.P., Klocke, J.A., Compadre, C.M., Hussain, R.A., Pezzuto, J.M.,Kinghorn, A.D., 1987. Characterization and feeding deterrent effects on the aphid, Schizaphis graminum, of some derivatives of the sweet compounds, stevioside and rebaudioside A. J. Nat. Prod. 50, 434-441.

Nikiforov, A.I., Eapen, A.K., 2008. A 90-day oral (dietary) toxicity study of rebaudioside A in Sprague-Dawley rats. Int. J. Toxicol. 27, 65-80.

Nunes, A.P., Ferreira-Machado, S.C., Nunes, R.M., Dantas, F.J., De Mattos, J.C., Caldiera de Araujo, A., 2007. Analysis of genotoxic potentiality of stevioside by comet assay. Food Chem. Toxicol., 45, 662-666.

Pezzuto, J.M., Compadre, C.M., Swanson, S.M., Nanayakkara, D., Kinghorn, A.D., 1985. Metabolically activated steviol, the aglycone of stevioside, is mutagenic. Proc. Natl. Acad. Sci. USA 82, 2478–2482.

Renwick, A.G.,1990. Acceptable daily intake and the regulation of intense sweeteners. Food Addit. Contam. 7, 463-75.

Renwick, A.G., 2008. The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. Food Chem. Toxicol., 46(Suppl. 1), S61-S69.

Roberts, A., Renwick, A.G., 2008. Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats. Food Chem. Toxicol., 46 (Suppl. 1), S31-S39.

Sekihashi, K., Saitoh, H., Sasaki, Y., 2002. Genotoxicity studies of Stevia extract and steviol by the comet assay. J. Toxicol. Sci. 27 (Suppl. 1), 1-8.

Sloter, E.D. 2008a. A Dietary Two-Generation Reproductive Toxicity Study of Chrysanta® 99-P in Rats. WIL Research Laboratories, LLC. Unpublished Report (Study Number WIL-568006).

Sloter, E.D., 2008b. Oral (Gavage) Study of Chrysanta® 99-P on Embryo/Fetal Development in Rats. WIL Research Laboratories, LLC. Unpublished Report (Study Number WIL-568004).

Soejarto, D.D., Kinghorn, A.D., Farnsworth, N.R., 1982. Potential sweetening agents of plant origin. III. Organoleptic evaluation of stevia leaf herbarium samples for sweetness. J. Nat. Prod., 45, 590-599.

Sunwin GRAS Notification for Rebaudioside A, 2010. Submitted to the US Food and Drug Administration, Washington, DC and identified as GRAS Notification 303. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=303.

Suttajit, M., Vinitketkaumnuen, U., Meevatee U.,Buddhasukh, D.,1993. Mutagenicity and human chromosomal effect of stevioside, a sweetener from Stevia rebaudiana Bertoni. Environ. Health Perspect.101, 53–56.

Switzerland Office of Public Health, 2008, http.www.bag.admin.ch/themen/lebensmittel/04861/04972/index.html?lang=fr.

The Food Institute Report, 2006. FDA News May 15, 2006. From Newsday, May 2, 2006.

Toyoda, K., Matsui, H., Shoda, T., Uneyama, C., Takahashi, M., 1997. Assessment of the carcinogenicity of stevioside in F344 rats. Food Chem. Toxicol., 35, 597–603.

Wagner, V.O., Van Dyke, M.R., 2006. Bacterial Reverse Mutation Assay of Rebaudioside A. BioReliance, Rockville, MD. Study Number AB21TG.503.BTL.

Wheeler, A., Boileau, A.C., Winkler, P.C., Compton, J.C., Prakash, I., Jiang, X., Mandarino, D.A., 2008. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. Food Chem. Toxicol., 46(7)(Suppl.), S54-S60.

WHO, 2000. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series; 42. Safety Evaluation of certain food additives. Stevioside.

WHO, 2003. GEMS/Food regional diets (regional per capita consumption of raw and semi processed agricultural commodities). Geneva: Global Environment Monitoring System 144 steviol glycosides K2 Food Contamination Monitoring and Assessment Programme and Food Safety Department, World Health Organization.

WHO, 2006. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series; 54. Safety evaluation of certain food additives, Steviol Glycosides, pp.117-144.

WHO, 2007. Joint FAO/WHO Expert Committee on Food Additives. 68th Meeting, Summary and Conclusions, Steviol Glycosides. Issued July 12, 2007.

WHO, 2008. Joint FAO/WHO Expert Committee on Food Additives. 69th Meeting, Summary and Conclusions, Steviol Glycosides. Issued July 4, 2008.

WHO, 2009. Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series: 60. Safety evaluation of certain food additives. Steviol Glycosides (addendum).

Williams, G. M., 2007. Letter to the Editor, Food Chem. Toxicol., 45, 2597-2598.

Williams, L.D., Burdock, G.A., 2009. Genotoxicity studies on a high-purity rebaudioside A preparation. Food Chem. Toxicol., 47, 1831-1836.

Xili, L., Chengjiany, B., Eryi, X., Reiming, S., Yuengming, W., Haodong, S., Zhiyian, H., 1992. Chronic oral toxicity and carcinogenicity study of stevioside in rats. Food Chem. Toxicol. 30, 957-965.

Zenith International, 2011. Stevia Sales Increased 27% Last Year, Says Zenith, http://www.nutritionaloutlook.com/news/stevia-sales-increased-27-last-year-says-zenith.

APPENDIX A

JECFA Steviol Glycosides Specifications & Analytical Method

Updated JECFA Specifications for Steviol Glycosides -- 2010

STEVIOL GLYCOSIDES

Prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property. Associated glycosides include rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside which are generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

Stevioside: 13-[(2-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy] kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

C.A.S. number

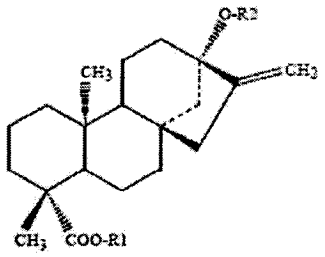
Stevioside: 57817-89-7
Rebaudioside A: 58543-16-1

Chemical formula

Stevioside: $C_{38}H_{60}O_{18}$
Rebaudioside A: $C_{44}H_{70}O_{23}$

Structural Formula

The nine named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α-Rha(2→1)
Rubusoside	β-Glc	β-Glc
Steviolbioside	H	β-Glc-β-Glc(2→1)

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides. Glc, Rha and Xyl represent, respectively, glucose, rhamnose and xylose sugar moieties.

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay	Not less than 95% of the total of the nine named steviol glycosides on the dried basis.
DESCRIPTION	White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.
FUNCTIONAL USES	Sweetener
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Freely soluble in water
Stevioside and rebaudioside A	The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)
PURITY	
Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under "General Methods, Metallic Impurities").
METHOD OF ASSAY	Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions. Reagents Acetonitrile: more than 95% transmittance at 210 nm. Standards Stevioside: more than 99.0% purity on the dried basis. Rebaudioside A: more than 99.0% purity on the dried basis. Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and

steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution
Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution
Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure
Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C_{18} MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.
Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%\textit{Rebaudioside A} = [W_R/W] \times [A_X/A_R] \times 100$$

where
X is each steviol glycoside;
W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
W is the amount (mg) calculated on the dried basis of sample in the sample solution;
A_S is the peak area for stevioside from the standard solution;
A_R is the peak area for rebaudioside from the standard solution;

A_X is the peak area of X for the sample solution; and
f_X is the ratio of the formula weight of X to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

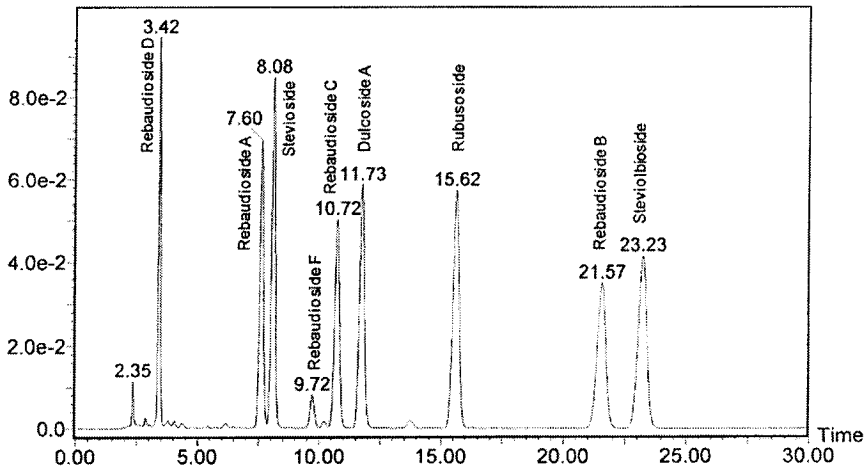


Figure. Chromatogram of mixture of nine steviol glycosides standard solution
Column: Capcell pak C_{18} MG II
Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

APPENDIX B

Manufacturing Information for Production of High Purity Rebaudioside A (≥ 95%)

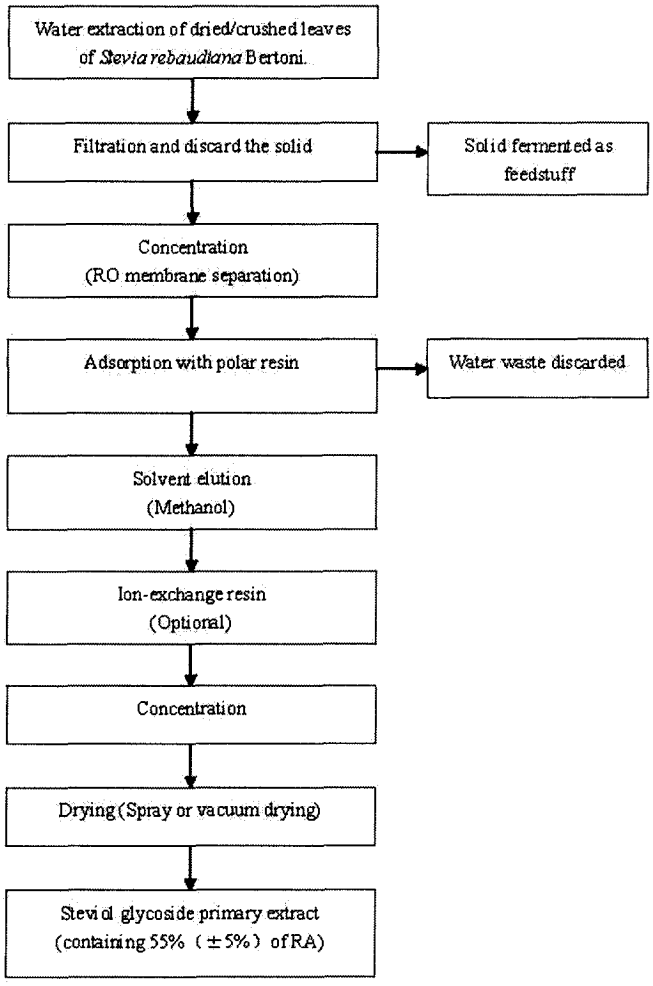
B-1 Process Flow Diagram for Steviol Glycosides Primary Extract

B-2 Process Flow Diagram for Preparation of Rebaudioside A

WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

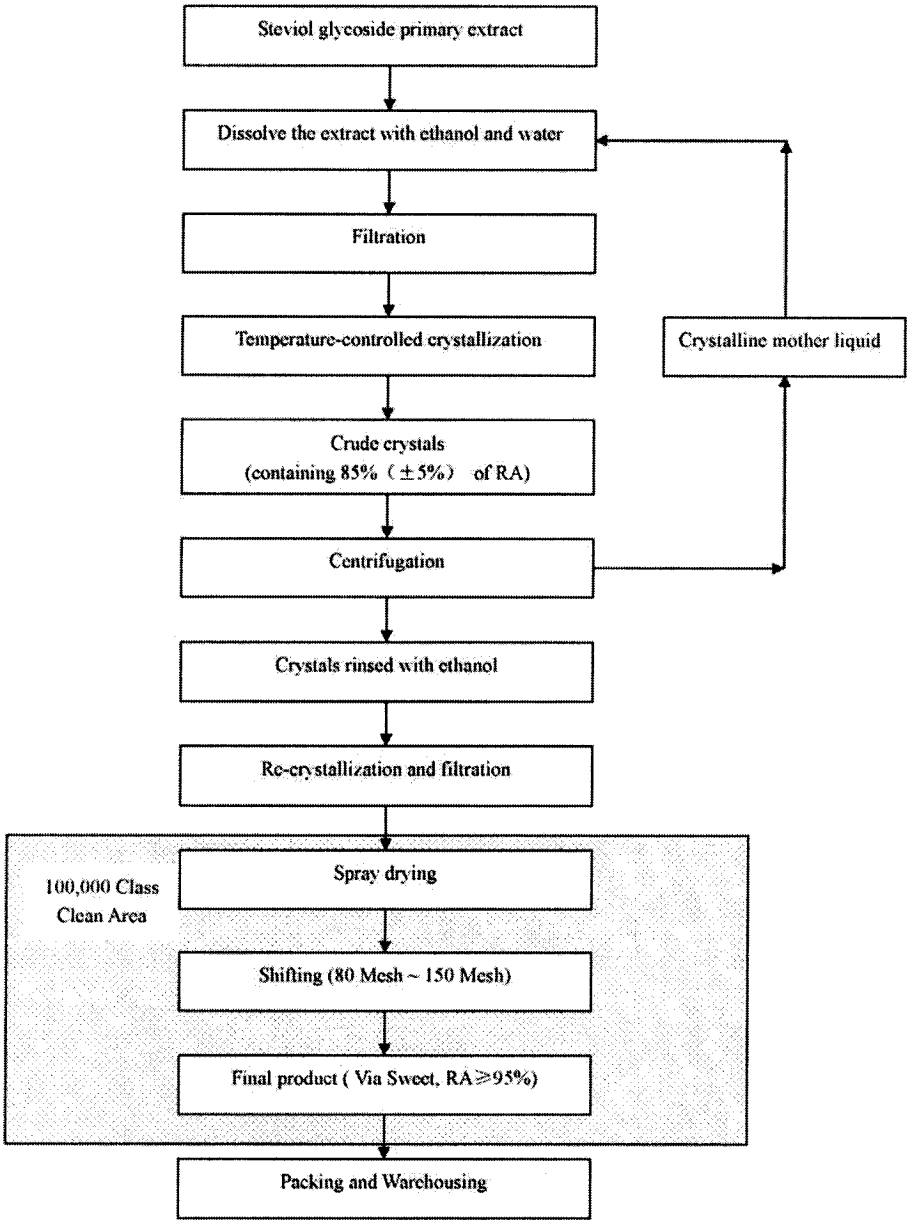
Manufacture Flow Chart of Rebaudioside A (Via Sweet)

First stage: Steviol glycoside primary extract



WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

Second stage: Purified Rebaudioside A product (Via Sweet)



BEST ORIGINAL COPY

APPENDIX C

Analyses of Multiple Production Lots of High Purity Rebaudioside A (≥ 95%)

Appendix C pages (268 with 4 dividers as listed below) not included in page number count.

Appendix C-1: Batch Analysis Data from Five Lots

Appendix C-2 Part 1: Analytical Report for Identity of Rebaudioside A
(Includes residual solvent levels from five bathes and pesticide residue analysis from one batch)

Appendix C-2 Part 2: Appendix 1 through 11, providing details of-

1. **Analytical Method**
2. **Calibration**
3. **Selectivity (Solvent Blank)**
4. **Accuracy/Selectivity (Positive Control)**
5. **Retention Time Markers**
6. **Reference Standard System Suitability**
7. **Sample Suitability**
8. **Reference Standards System Suitability (USP Tailing Factor, Theoretical Plates)**
9. **Accuracy**
10. **Repeatability, Precision, Results (Sample Chromatogram)**
11. **Rebaudioside D & F Chromatograms**

Appendix C-3: Pesticide Residue Analyses for High Purity Rebaudioside A

APPENDIX C-1

Batch Analysis Data from Five Lots

WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

Certificate Of Analysis

NO: C-041-10-009

Product name	Rebaudioside A		
Batch number	Z 100202	**Batch quantity**	725.60 kg
Test Date	February 3, 2010	**Manufacturing date**	February 3, 2010
Certificate Date	February 6, 2010	**Expiry date**	February 3, 2012

ITEMS	SPECIFICATION	RESULTS
PHYSICAL TESTS:		
Description		
Appearance	White Powder	Complies
Odor	Characteristic	Complies
Taste	Characteristic	Complies
Particle size	100% through 80 mesh	Complies
Solubility	Soluble in water	Complies
CHEMICAL TESTS:		
Rebaudioside A (wt/wt% on dry basis)	≥95%	97.54%
Related Steviol Glycosides (wt/wt% on dry basis)	≤5.0%	2.52%
Total Steviol Glycosides (wt/wt% on dry basis)	≥95%	100.06%
Moisture Content (wt/wt% by Loss on Drying)	≤5.0%	3.36%
Sulfated ash	≤1.0%	0.12%
Arsenic (As)	≤1mg/kg	0.10mg/kg
Lead (Pb)	≤1mg/kg	0.01mg/kg
PH(1 in 100 solution)	Between 4.5 and 7.0	5.52
Residual solvents		
MeOH	≤200mg/kg	61.62mg/kg
EtOH	≤5000mg/kg	211.60mg/kg
MICROBIOLOGY TESTS		
Total Aerobic Plate Count	≤1000cfu/g	<10cfu/g
Total Aerobic Mold Count	≤100cfu/g	<10cfu/g
Total Aerobic Yeast Count	≤100cfu/g	<10cfu/g
Coliform/E.Coli	Negative/g	Negative/g

Conclusion Conform to specification. (By Wagott criteria for Rebaudioside A)

Storage Store 15℃-25℃. Keep away from strong light and heat.

Shelf life 2 Years when properly stored

000061

(b) (6)

Guo Qiaom

Quality Control Ma

WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

Certificate Of Analysis

NO: C-041-10-010

Product name	Rebaudioside A		
Batch number	Z 100203	**Batch quantity**	546.00 kg
Test Date	February 4, 2010	**Manufacturing date**	February 4, 2010
Certificate Date	February 7, 2010	**Expiry date**	February 4, 2012

ITEMS	SPECIFICATION	RESULTS
PHYSICAL TESTS:		
Description		
Appearance	White Powder	Complies
Odor	Characteristic	Complies
Taste	Characteristic	Complies
Particle size	100% through 80 mesh	Complies
Solubility	Soluble in water	Complies
CHEMICAL TESTS:		
Rebaudioside A (wt/wt% on dry basis)	≥95%	97.56%
Related Steviol Glycosides (wt/wt% on dry basis)	≤5.0%	2.56%
Total Steviol Glycosides (wt/wt% on dry basis)	≥95%	100.12%
Moisture Content (wt/wt% by Loss on Drying)	≤5.0%	3.27%
Sulfated ash	≤1.0%	0.15%
Arsenic (As)	≤1mg/kg	0.09mg/kg
Lead (Pb)	≤1mg/kg	0.01mg/kg
PH(1 in 100 solution)	Between 4.5 and 7.0	5.68
Residual solvents		
MeOH	≤200mg/kg	96.02mg/kg
EtOH	≤5000mg/kg	254.18mg/kg
MICROBIOLOGY TESTS		
Total Aerobic Plate Count	≤1000cfu/g	<10cfu/g
Total Aerobic Mold Count	≤100cfu/g	<10cfu/g
Total Aerobic Yeast Count	≤100cfu/g	<10cfu/g
Coliform/E.Coli	Negative/g	Negative/g

Conclusion Conform to specification. (By Wagott criteria for Rebaudioside A)

Storage Store 15℃-25℃. Keep away from strong light and heat.

Shelf life 2 Years when properly stored

000062

(b) (6)

Guo Qiaoni

Quality Control Manager

WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

Certificate Of Analysis

NO: C-041-10-023

Product name	Rebaudioside A		
Batch number	Z 100301	**Batch quantity**	565.70 kg
Test Date	March 1, 2010	**Manufacturing date**	March 1, 2010
Certificate Date	March 4, 2010	**Expiry date**	March 1, 2012

ITEMS	SPECIFICATION	RESULTS
PHYSICAL TESTS:		
Description		
Appearance	White Powder	Complies
Odor	Characteristic	Complies
Taste	Characteristic	Complies
Particle size	100% through 80 mesh	Complies
Solubility	Soluble in water	Complies
CHEMICAL TESTS:		
Rebaudioside A (wt/wt% on dry basis)	≥95%	97.49%
Related Steviol Glycosides (wt/wt% on dry basis)	≤5.0%	2.58%
Total Steviol Glycosides (wt/wt% on dry basis)	≥95%	100.07%
Moisture Content (wt/wt% by Loss on Drying)	≤5.0%	3.02%
Sulfated ash	≤1.0%	0.12%
Arsenic (As)	≤1mg/kg	0.09mg/kg
Lead (Pb)	≤1mg/kg	0.01mg/kg
PH(1 in 100 solution)	Between 4.5 and 7.0	5.64
Residual solvents		
MeOH	≤200mg/kg	189.14mg/kg
EtOH	≤5000mg/kg	221.55mg/kg
MICROBIOLOGY TESTS		
Total Aerobic Plate Count	≤1000cfu/g	<10cfu/g
Total Aerobic Mold Count	≤100cfu/g	<10cfu/g
Total Aerobic Yeast Count	≤100cfu/g	<10cfu/g
Coliform/E.Coli	Negative/g	Negative/g

Conclusion Conform to specification. (By Wagott criteria for Rebaudioside A)

Storage Store 15℃-25℃. Keep away from strong light and heat.

Shelf life 2 Years when properly stored

000063

(b) (6)

Guo Qiaon

Quality Control Ma

WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

Certificate Of Analysis

NO: C-041-10-024

Product name	Rebaudioside A		
Batch number	Z 100302	**Batch quantity**	518.51 kg
Test Date	March 1, 2010	**Manufacturing date**	March 1, 2010
Certificate Date	March 4, 2010	**Expiry date**	March 1, 2012

ITEMS	SPECIFICATION	RESULTS
PHYSICAL TESTS:		
Description		
Appearance	White Powder	Complies
Odor	Characteristic	Complies
Taste	Characteristic	Complies
Particle size	100% through 80 mesh	Complies
Solubility	Soluble in water	Complies
CHEMICAL TESTS:		
Rebaudioside A (wt/wt% on dry basis)	≥95%	97.40%
Related Steviol Glycosides (wt/wt% on dry basis)	≤5.0%	2.68%
Total Steviol Glycosides (wt/wt% on dry basis)	≥95%	100.08%
Moisture Content (wt/wt% by Loss on Drying)	≤5.0%	3.19%
Sulfated ash	≤1.0%	0.10%
Arsenic (As)	≤1mg/kg	0.13mg/kg
Lead (Pb)	≤1mg/kg	0.02mg/kg
PH(1 in 100 solution)	Between 4.5 and 7.0	5.61
Residual solvents		
MeOH	≤200mg/kg	129.82mg/kg
EtOH	≤5000mg/kg	217.31mg/kg
MICROBIOLOGY TESTS		
Total Aerobic Plate Count	≤1000cfu/g	<10cfu/g
Total Aerobic Mold Count	≤100cfu/g	<10cfu/g
Total Aerobic Yeast Count	≤100cfu/g	<10cfu/g
Coliform/E.Coli	Negative/g	Negative/g

Conclusion Conform to specification. (By Wagott criteria for Rebaudioside A)

Storage Store 15℃-25℃. Keep away from strong light and heat.

Shelf life 2 Years when properly stored

000064

(b) (6)

Guo Qiaoni

Quality Control Ma

WAGOTT CHENGDU WAGOTT PHARMACEUTICAL CO.,LTD.

Certificate Of Analysis

NO: C-041-10-037

Product name	Rebaudioside A		
Batch number	Z 100401	**Batch quantity**	899.25 kg
Test Date	April 3, 2010	**Manufacturing date**	April 3, 2010
Certificate Date	April 6, 2010	**Expiry date**	April 3, 2012

ITEMS	SPECIFICATION	RESULTS
PHYSICAL TESTS:		
Description		
Appearance	White Powder	Complies
Odor	Characteristic	Complies
Taste	Characteristic	Complies
Particle size	100% through 80 mesh	Complies
Solubility	Soluble in water	Complies
CHEMICAL TESTS:		
Rebaudioside A (wt/wt% on dry basis)	≥95%	97.14%
Related Steviol Glycosides (wt/wt% on dry basis)	≤5.0%	2.98%
Total Steviol Glycosides (wt/wt% on dry basis)	≥95%	100.12%
Moisture Content (wt/wt% by Loss on Drying)	≤5.0%	3.26%
Sulfated ash	≤1.0%	0.10%
Arsenic (As)	≤1mg/kg	0.18mg/kg
Lead (Pb)	≤1mg/kg	0.01mg/kg
PH(1 in 100 solution)	Between 4.5 and 7.0	5.52
Residual solvents		
MeOH	≤200mg/kg	37.20mg/kg
EtOH	≤5000mg/kg	257.18mg/kg
Organchlorine pesticide residues		
BHC	≤0.1mg/kg	Complies
DDT	≤0.1mg/kg	Complies
PCNB	≤0.1mg/kg	Complies
MICROBIOLOGY TESTS		
Total Aerobic Plate Count	≤1000cfu/g	<10cfu/g
Total Aerobic Mold Count	≤100cfu/g	<10cfu/g
Total Aerobic Yeast Count	≤100cfu/g	<10cfu/g
Coliform/E.Coli	Negative/g	Negative/g

000065

Conclusion Conform to specification. (By Wagott criteria for Rebaudioside A)

Storage Store 15℃-25℃. Keep away from strong light and heat.

Shelf life 2 Years when properly stored

(b) (6)

000065

Guo Qiaom

Quality Control M

APPENDIX C-2 Part 1

Analytical Report for Identity of Rebaudioside A

Eurofins Scientific, Inc.
1365 Redwood Way
Petaluma, Ca 94951

Method Verification for the Determination of Rebaudioside A and Related Steviol Glycosides by High Performance Liquid Chromatography (HPLC) with Purity and Solvent Analysis of 5 Production Samples and Selected Pesticide Screening

(This report contains updates for clarification and supersedes the December 2010 report.)

Prepared by: (b) (6)
Jules Skamarack
Eurofins Scientific, Inc.

Reviewed by: (b) (6)
Mariel Esguerra
Eurofins Scientific, Inc.

Approved by: _____
Mathew Titlow
Chengdu Wagott Pharmaceutical Co., Ltd.

Date Issued: March 2011

I. Study Identification

1. Study Title:
Method verification for the determination of rebaudioside A and related steviol glycosides by high performance liquid chromatography (HPLC) with purity and solvent residue analysis of 5 production samples and selected pesticide screening

2. Study Objective:
The objective of this study is to verify the assay for rebaudioside A and related glycosides as listed in JEFCA 2008 for 5 lots of *Rebaudioside A 95%* submitted from Chengdu Wagott Pharmaceutical Co., Ltd. Additionally the study assayed for the concentrations of residual methanol (MeOH) and ethanol (EtOH) in the 5 lots. One lot was tested for levels of residual pesticides. Methods are listed in the following 3 tables:

Eurofins Method #	Pesticide List	Method Citation	Instrumentation and Detectors
QA017	Luke II Organochlorine	FDA PAM 302 E7C6	GC-ECD
QA018	Luke II Pyrethroids	FDA PAM 302 E7C6	GC-ECD
QA019	Luke II Organonitrogen	FDA PAM 302 E7C6	GC-MS
QA01A	Luke II Organophosphorus	FDA PAM 302 E7C6	GC-FPD
QA01B	Luke II Carbamates	FDA PAM 302 E7C6	LC-MSMS

Eurofins Method #	Solvent Tested	Method Citation	Instrumentation and Detectors
QA256	Residual Ethanol	USP/NF <467>	GC-MS
QA 367	Residual Methanol	USP <467>	GC-MS

Eurofins Method #	Compounds Tested	Method Citation	Instrumentation and Detectors
KK149	Steviol Glycosides	JECFA 2008 modified	HPLC-UV

Data and methodology are provided below to conform to the verification study of Rebaudioside A analysis.

3. Study Coordinator/Performing Laboratory:
Jules Skamarack, Eurofins Scientific, Inc.

4. Study Monitor(s):
Mariel Esguerra, Eurofins Scientific, Inc.

5. Test Materials:
Stevia rebaudiana Leaf extracts

(1) Rebaudioside A, Lot (b) (4) , Eurofins sample number 740-2010-00007946

(2) Rebaudioside A, Lot (b) (4) , Eurofins sample number 740-2010-00007947

(3) Rebaudioside A, Lot (b) (4) , Eurofins sample number 740-2010-00007948

(4) Rebaudioside A, Lot (b) , Eurofins sample number 740-2010-00007949

(5) Rebaudioside A, Lot # (b) , Eurofins sample number 740-2010-

6. Test Reagents:

(1) Acetonitrile, HPLC Grade
(2) Fisher P/N A998-4, VWR P/N JT9017-3
(3) Rebaudioside A Reference Material, Lot. F01077 from USP Catalogue # 1600121, C.A.S # 58543-16-1
(4) Positive control sample for day to day accuracy check identified as Eurofins control # LCKK149-2 monitored for rebaudioside A concentration.
(5) Phosphoric Acid, Fischer Chemical Company P/N A260
(6) Steviolbioside Reference Material, Lot # 19349-1821 from ChromaDex Catalogue # 19349010, CAS # 41093-60-1
(7) Rebaudioside B Reference Material, Lot # Lot # 18227-101 from ChromaDex Catalogue # ASB00018227, CAS # 58543-17-2
(8) Stevioside Reference Material, Lot # F01080 from USP Catalogue # 1622408, CAS # 471-80-7
(9) Rebaudioside C Reference Material, Lot # 00018228-3202 from ChromaDex Catalogue # ASB-00018228, CAS # 63550-99-2

Mobile Phase Preparation: (see attached method)

7. Method References:

High Performance Liquid Chromatographic Determination of Individual Sweet Diterpenoid Glycosides of *Stevia rebaudiana*, W.A.Court, Agriculture & Food Canada Pest Management Research Centre, P.O. Box 186, Ontario, N4B 2W9

Steviol glycosides, Prepared at the 69th JEFCA (2008) published in FAO JECFA Monographs 5 (2008) superseding specification prepared in the 68th JEFCA (2007), published in FAO JECFA Monographs 5 (2008). An ADI of 0-4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

II. Study Description

1. Scope:

This is applicable to the determination of rebaudioside A, stevioside and Stevia glycosides in 5 raw material samples.

2. Test Materials:

(1) Rebaudioside A, Lot # (b) (4) , Eurofins sample number 740-2010-00007946
(2) Rebaudioside A, Lot (b) (4) , Eurofins sample number 740-2010-00007947
(3) Rebaudioside A, Lot (b) (4) , Eurofins sample number 740-2010-00007948

(4) Rebaudioside A, Lot #(b) (4), Eurofins sample number 740-2010-00007949

(5) Rebaudioside A, Lot #(b) (4), Eurofins sample number 740-2010-00007950

3. Reference Standards: (rebaudioside A)

A. Stock standards:

1. The reference material was preparation for rebaudioside A by drying at 105 degrees Centigrade for two hours as directed by JECFA.
2. On a microbalance, accurately weigh 10.0 ± 1 mg of rebaudioside A USP reference material; quantitatively transfer to a 5-mL volumetric flask with mobile phase. Dissolve using heat if necessary. Cool to room temperature and dilute to volume with mobile phase. Concentration is approximately 2 mg/mL rebaudioside A.
3. Steviolbioside, rebaudioside B, stevioside and rebaudioside C reference materials were run as qualitative retention time markers. A sufficient amount of each material to create a detectable peak from the method was placed in an injection vial and solublized with mobile phase.

B. Reference standard preparation (USP rebaudioside A). A single point calibration is used per JECFA for determination of high purity samples. To accommodate this, the stock reference material is used as prepared. The actual concentration is adjusted for standard purity and is listed below for rebaudioside A:

Reference Standard Concentration (mg/ml)
2.0893578

4. Verification Study:

A. Primary method LC-KK149.00: (method is attached in **appendix #1**)

B. Single Point Calibration:

1. A single point calibration is used by injecting the reference stock standard a minimum of five times. This is a modification from the attached method used to achieve greater accuracy on high purity analyses.
   a. Calculate response factors. RSD between injections must be ≤1.5%.
2. **Results, rebaudioside A;**
   a. Response factors RSD between levels (amount) was found to be 0.0107614 and passed the criteria (appendix #2).

C. Selectivity: For purposes of this study, selectivity is specificity

1. Preparation solvent blank analysis:
   a. Preparation solvent blanks are to be free of peaks.
      ii. Result: Blanks were shown to be free of peaks. (file # 2010-10-18\1FA-0101.D and -0102D) (appendix # 3)
2. Positive control and accuracy sample analysis:
   a. Analyze the positive control sample for the determination of accuracy and peak separation. The positive control is tested with each analytical run to verify concentration and proper dilution of the reference material into the reference standard for accuracy of the determination. The result of the positive control is monitored

using control charting. The measured concentration of rebaudioside A should be with-in 2 standard deviations of the mean. The mean result is established at 64.0 %(w/w) and the standard deviation is 0.281. This control was analyzed twice labeled as *control new* and *control old*, based upon where they stood in the analysis sequence.

ii. Result: Measured concentration results are 63.999018 and 64.473495 % (w/w) which passed the criteria. (file #'s 2010-10-18\1FE-0601.D and 2010-10-18\1BD-5001.D) (Appendix # 4)

3. Demonstrate separation of the two major peaks, stevioside and rebaudioside A in the positive control:

a. Separation was demonstrated with actual retention times of 3.976 minutes (stevioside) and 5.063 minutes (rebaudioside A) (file # 2010-10-18\1FE-0601.D) (Appendix # 4)

4. Retention time markers were tested to verify the retention time of the following related glycosides in both standards and samples; steviolbioside, rebaudioside B, Stevioside and rebaudioside C. **(Appendix # 5)**

D. System Suitability:

1. The reference stock standard 2.089357 mg/ml rebaudioside A solution is injected after every five to six sample injections and at the end of the analysis sequence.

a. Acceptance criteria: The system is considered suitable if the retention times of the standard peaks do not deviate more than 0.5 minutes and the RSD of the peak areas are less than 2%.

ii. Results: Average retention time 5.066 minutes with a RSD of 0.095. The actual deviation between the longest and shortest retention time is 0.019 (5.077 minutes to 5.058 minutes) minutes. All criteria pass. (Appendix # 6)

2. USP tailing factor was determined for the rebaudioside A peak from the sample matrix. The tailing factor should be not more than (NMT) 2.0. The tailing factors from the run labeled A of each sample are listed below:

Sample	USP Tailing Factor
7946A	0.958
7947A	0.958
7948A	0.956
7949A	0.959
7950A	0.955

a. Results: The tailing factor passed the criteria (USP) at 0.957 (average). (Appendix # 7)

3. Column Efficiency, Not less than (NLT) 5000 theoretical plate count, using the Rebaudioside A peak from the sample matrix.

Sample	Plates (Halfwidth method)
7946A	7268
7947A	7491
7948A	7491
7949A	7484
7950A	7242

a. Results: All theoretical plate count calculations were greater than 5000 with the halfwidth **method calculating the average of all samples at 7395. All pass the criteria. (Appendix # 7)**

4. USP tailing factor was determined for the rebaudioside A standard peak at the 2.0934276 mg/ml concentration and was determined on 12 injections. The tailing factor should be not more than (NMT) 2.0.

a. Results: The tailing factor passed the criteria (USP) at 0.954. (Appendix # 8)

5. Column efficiency, Not less than (NLT) 5000 theoretical plate count, using the rebaudioside A standard peak at the 2.0934276 mg/ml concentration and was determined on 12 injections.

a. Results: All theoretical plate count calculations were greater than 5000 with the halfwith method calculating at 7356 (average). All pass the criteria. (Appendix # 8)

E. Accuracy:

1. Accuracy was examined in the initial validation for the method KK149. For purposes of this study, the USP reference standard was compared to the Eurofins control sample LCKK149-2. Accuracy based on the analysis of the control sample passes the criteria. See section 4C2 and **appendix 4.** Additionally, Section F (Accuracy) from the LC-KK149.00 method validation report is included with example chromatograms **(Appendix # 9)**

F. Repeatability and Precision:

1. For each sample, perform 3 replicate sample preparations. The sample assay preparation concentration was diluted to approximately 2 mg/ml to match the concentration of the rebaudioside reference standard and was based on the expected test sample concentration. All samples were dried to the anhydrous solvent free basis at 105 degrees Centigrade for 2 hours as directed by JECFA. The acceptance criteria for the relative standard deviation (RSD) for each set of 3 analyses must be $\leq$ 5% for the total steviol glycoside value.

a. Results: As seen in the following table, RSDs for rebaudioside A on all samples pass the criteria. In addition all measurements confirmed a minimum passing specification for rebaudioside A of 95% dry weight, solvent free basis. The retention time of the peak rebaudioside A in the chromatogram of the assay preparation corresponds to that in the chromatogram of the reference standard preparation as obtained during the Assay confirming the identification of the material. Test material sample chromatograms are located in appendix # 10.

Sample 7946	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.034601	0.036221	0.039898	0.0369067	7.354364
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.2809	1.2849	1.2729	1.2795667	0.4775133
Stevioside	0.97177	0.94766	0.95604	0.95849	1.2770397
Rebaudioside C	0.76694	0.77745	0.77369	0.7726933	0.6892015
Unknown	0.60265	0.61755	0.6269	0.6157	1.9864207
Rebaudioside A	95.017	95.019	95.041	95.025667	**0.0140137**
Total	98.674	98.682	98.711	98.689	0.0197265

Sample 7947	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.030008	0.0370072	0.034937	0.0339841	10.580221
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.3065	1.2986	1.3062	1.3037667	0.343388
Stevioside	1.0763	1.0699	1.0965	1.0809	1.284467
Rebaudioside C	0.81024	0.82954	0.82565	0.82181	1.2420077
Unknown	0.63254	0.62296	0.65837	0.6379567	2.8710259
Rebaudioside A	97.977	97.719	97.603	97.766333	**0.195814**
Total	101.82	101.58	101.52	101.64	0.1561837

Sample 7948	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.034792	0.036266	0.035551	0.0355363	2.0742417
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.2956	1.3111	1.2726	1.2931	1.4980567
Stevioside	1.0011	1.0308	1.0009	1.0109333	1.7019251
Rebaudioside C	0.89892	0.91213	0.9019	0.9043167	0.7661755
Unknown	0.71476	0.77668	0.71965	0.73703	4.6707521
Rebaudioside A	97.505	97.096	97.014	97.205	**0.2705857**
Total	101.45	101.16	100.49	101.03333	0.4873394
Sample 7949	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.035686	0.030632	0.032974	0.0330973	7.6418733
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.4687	1.4678	1.5188	1.4851	1.9654249
Stevioside	1.0309	1.0364	1.0861	1.0511333	2.8927475
Rebaudioside C	0.90192	0.93049	0.97317	0.9351933	3.8341912
Unknown	0.74674	0.70001	0.78405	0.7436	5.6627082
Rebaudioside A	97.533	97.104	97.090	97.242333	**0.2589634**
Total	101.72	101.27	101.49	101.49333	0.2217077

Sample 7950	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.039603	0.035764	0.03232	0.035046	10.395722
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.5119	1.4648	1.4699	1.4822	1.7438301
Stevioside	1.2482	1.2486	1.2437	1.2468333	0.2182254
Rebaudioside C	0.90182	0.96317	0.97761	0.9475333	4.2470162
Unknown	0.75445	0.74629	0.75924	0.7533267	0.8691679
Rebaudioside A	97.437	97.232	97.101	97.256667	**0.1741296**
Total	101.89	101.69	101.58	101.72	0.1545049

G. Analysis of residual solvents for ethanol and methanol was performed following USP <467> criteria. Results are listed below with the accompanying USP and JEFCA specification for these residuals. **All samples pass the criteria.**

	Result (mg/kg)		JECFA Specification (mg/kg)		USP/FCC Specification (mg/kg)	
Sample	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
7946	<50	90	NMT 200	NMT 5000	NMT 200	NMT 5000
7947	<50	270	NMT 200	NMT 5000	NMT 200	NMT 5000
7948	82	67	NMT 200	NMT 5000	NMT 200	NMT 5000
7949	<50	78	NMT 200	NMT 5000	NMT 200	NMT 5000
7950	<50	84	NMT 200	NMT 5000	NMT 200	NMT 5000

H. Analysis of pesticide residuals was also performed following the criteria listed above. The samples were found to be free of pesticides (none detected ND) at the listed limits of quantitation (LOQ) in the table below.

Sample 7946 Pesticide Screen FDA PAM 302 E7C6 (mg/kg)			
Screen	Pesticide	Result	LOQ
Pesticides - Luke II Carbamates	Oxamyl	ND	0.02
Pesticides - Luke II Carbamates	Propoxur	ND	0.02
Pesticides - Luke II Carbamates	Thiodicarb	ND	0.02
Pesticides - Luke II Carbamates	Methiocarb	ND	0.02
Pesticides - Luke II Carbamates	Methomyl	ND	0.02
Pesticides - Luke II Carbamates	o-Phenylphenol	ND	0.02
Pesticides - Luke II Carbamates	Carbaryl	ND	0.02
Pesticides - Luke II Carbamates	Carbofuran	ND	0.02
Pesticides - Luke II Carbamates	Carbofuran 3-OH	ND	0.02
Pesticides - Luke II Carbamates	Aldicarb	ND	0.02
Pesticides - Luke II Carbamates	Aldicarb sulfone	ND	0.02
Pesticides - Luke II Carbamates	Aldicarb sulfoxide	ND	0.02
Pesticides - Luke II Organophosphorus	Thionazin	ND	0.02
Pesticides - Luke II Organophosphorus	Propetamphos	ND	0.02
Pesticides - Luke II Organophosphorus	Ronnel	ND	0.02
Pesticides - Luke II Organophosphorus	Tetrachlorvinphos	ND	0.02
Pesticides - Luke II Organophosphorus	Phosphamidon	ND	0.02
Pesticides - Luke II Organophosphorus	Pirimiphos-methyl	ND	0.02
Pesticides - Luke II Organophosphorus	Profenofos	ND	0.02
Pesticides - Luke II Organophosphorus	Phorate	ND	0.02
Pesticides - Luke II Organophosphorus	Phosalone	ND	0.02
Pesticides - Luke II Organophosphorus	Phosmet	ND	0.02
Pesticides - Luke II Organophosphorus	Mevinphos	ND	0.02
Pesticides - Luke II Organophosphorus	Omethoate	ND	0.02
Pesticides - Luke II Organophosphorus	Parathion	ND	0.02
Pesticides - Luke II Organophosphorus	Methamidophos	ND	0.02
Pesticides - Luke II Organophosphorus	Methidathion	ND	0.02

Pesticides - Luke II Organophosphorus	Methyl Parathion	ND	0.02
Pesticides - Luke II Organophosphorus	Isofenphos	ND	0.02
Pesticides - Luke II Organophosphorus	Malathion	ND	0.02
Pesticides - Luke II Organophosphorus	Metasystox-R	ND	0.02
Pesticides - Luke II Organophosphorus	Fenitrothion	ND	0.02
Pesticides - Luke II Organophosphorus	Fenthion	ND	0.02
Pesticides - Luke II Organophosphorus	Fonofos	ND	0.02
Pesticides - Luke II Organophosphorus	Ethion	ND	0.02
Pesticides - Luke II Organophosphorus	Ethoprop	ND	0.02
Pesticides - Luke II Organophosphorus	Fenamiphos	ND	0.02
Pesticides - Luke II Organophosphorus	Dimethoate	ND	0.02
Pesticides - Luke II Organophosphorus	Disulfoton	ND	0.02
Pesticides - Luke II Organophosphorus	EPN	ND	0.02
Pesticides - Luke II Organophosphorus	Diazinon	ND	0.02
Pesticides - Luke II Organophosphorus	Dibrom (Naled)	ND	0.02
Pesticides - Luke II Organophosphorus	Dicrotophos	ND	0.02
Pesticides - Luke II Organophosphorus	Coumaphos	ND	0.02
Pesticides - Luke II Organophosphorus	DEF	ND	0.02
Pesticides - Luke II Organophosphorus	Demeton group	ND	0.02
Pesticides - Luke II Organophosphorus	Chlorpyrifos	ND	0.02
Pesticides - Luke II Organophosphorus	Chlorpyrifos-methyl	ND	0.02
Pesticides - Luke II Organophosphorus	Ciodrin (Crotoxyphos)	ND	0.02
Pesticides - Luke II Organophosphorus	Bensulide	ND	0.02
Pesticides - Luke II Organophosphorus	Carbophenothion	ND	0.02
Pesticides - Luke II Organophosphorus	Chlorfenvinphos	ND	0.02
Pesticides - Luke II Organonitrogen	Simazine	ND	0.02
Pesticides - Luke II Organonitrogen	Tebuconazole	ND	0.02
Pesticides - Luke II Organonitrogen	Terbacil	ND	0.02
Pesticides - Luke II Organonitrogen	Pymetrozine	ND	0.02
Pesticides - Luke II Organonitrogen	Pyraclostrobin	ND	0.02
Pesticides - Luke II Organonitrogen	Pyriproxyfen	ND	0.02
Pesticides - Luke II Organonitrogen	Prometon	ND	0.02
Pesticides - Luke II Organonitrogen	Prometryn	ND	0.02
Pesticides - Luke II Organonitrogen	Propamocarb	ND	0.02
Pesticides - Luke II Organonitrogen	Metalaxyl	ND	0.02
Pesticides - Luke II Organonitrogen	Metolachlor	ND	0.02
Pesticides - Luke II Organonitrogen	Molinate	ND	0.02
Pesticides - Luke II Organonitrogen	Hexazinone	ND	0.02
Pesticides - Luke II Organonitrogen	Imazalil	ND	0.02
Pesticides - Luke II Organonitrogen	Kresoxim-methyl	ND	0.02
Pesticides - Luke II Organonitrogen	Fenamidone	ND	0.02
Pesticides - Luke II Organonitrogen	Fipronil	ND	0.02
Pesticides - Luke II Organonitrogen	Fludioxonil	ND	0.02
Pesticides - Luke II Organonitrogen	Cyprodinil	ND	0.02
Pesticides - Luke II Organonitrogen	Dimethomorph	ND	0.02
Pesticides - Luke II Organonitrogen	Diphenylamine	ND	0.02
Pesticides - Luke II Organonitrogen	Benthiocarb	ND	0.02
Pesticides - Luke II Organonitrogen	Cyanazine	ND	0.02
Pesticides - Luke II Organonitrogen	Cyromazine	ND	0.02
Pesticides - Luke II Organonitrogen	Acetamiprid	ND	0.02
Pesticides - Luke II Organonitrogen	Atrazine	ND	0.02
Pesticides - Luke II Organonitrogen	Azoxystrobin	ND	0.02
Pesticides - Luke II Pyrethroids	Tralomethrin	ND	0.02
Pesticides - Luke II Pyrethroids	Fluvalinate	ND	0.02
Pesticides - Luke II Pyrethroids	Cyhalothrin lambda	ND	0.02
Pesticides - Luke II Pyrethroids	Permethrin	ND	0.02
Pesticides - Luke II Pyrethroids	Deltamethrin	ND	0.02
Pesticides - Luke II Pyrethroids	Fenpropathrin	ND	0.02
Pesticides - Luke II Pyrethroids	Esfenvalerate	ND	0.02
Pesticides - Luke II Pyrethroids	Bifenthrin	ND	0.02
Pesticides - Luke II Pyrethroids	Cyfluthrin	ND	0.02
Pesticides - Luke II Pyrethroids	Cypermethrin	ND	0.02
Pesticides - Luke II Organochlorine	Pyrethrins (sum of 6)	ND	0.02
Pesticides - Luke II Organochlorine	HCB (Hexachlorbenzene)	ND	0.01
Pesticides - Luke II Organochlorine	Metribuzin	ND	0.01
Pesticides - Luke II Organochlorine	Myclobutanil	ND	0.01
Pesticides - Luke II Organochlorine	Vegadex	ND	0.02
Pesticides - Luke II Organochlorine	Vinclozolin	ND	0.02
Pesticides - Luke II Organochlorine	Chlorobenzilate	ND	0.04
Pesticides - Luke II Organochlorine	Trifloxystrobin	ND	0.01

Pesticides - Luke II Organochlorine	Triflumizole	ND	0.01
Pesticides - Luke II Organochlorine	Trifluralin	ND	0.02
Pesticides - Luke II Organochlorine	Tetradifon	ND	0.02
Pesticides - Luke II Organochlorine	Toxaphene (camphechlor)	ND	0.01
Pesticides - Luke II Organochlorine	Tridimephon	ND	0.02
Pesticides - Luke II Organochlorine	Profluralin	ND	0.02
Pesticides - Luke II Organochlorine	Pronamide	ND	0.02
Pesticides - Luke II Organochlorine	Propanil	ND	0.01
Pesticides - Luke II Organochlorine	Perthane	ND	0.01
Pesticides - Luke II Organochlorine	Polychlorinated Biphenyls	ND	0.01
Pesticides - Luke II Organochlorine	Procymidone	ND	0.01
Pesticides - Luke II Organochlorine	Pendimethalin	ND	0.01
Pesticides - Luke II Organochlorine	Pentachloronitrobenzene (PCNB)	ND	0.01
Pesticides - Luke II Organochlorine	Pentachloroaniline	ND	0.01
Pesticides - Luke II Organochlorine	Mirex	ND	0.01
Pesticides - Luke II Organochlorine	Oxadiazon	ND	0.02
Pesticides - Luke II Organochlorine	Oxyfluorfen	ND	0.01
Pesticides - Luke II Organochlorine	Lindane (gamma-HCH)	ND	0.01
Pesticides - Luke II Organochlorine	Linuron	ND	0.1
Pesticides - Luke II Organochlorine	Methoxychlor	ND	0.02
Pesticides - Luke II Organochlorine	Heptachlor epoxide	ND	0.01
Pesticides - Luke II Organochlorine	Indoxacarb	ND	0.02
Pesticides - Luke II Organochlorine	Iprodione	ND	0.02
Pesticides - Luke II Organochlorine	Fenhexamid	ND	0.02
Pesticides - Luke II Organochlorine	Folpet	ND	0.02
Pesticides - Luke II Organochlorine	Heptachlor	ND	0.01
Pesticides - Luke II Organochlorine	Endosulfan-sulfate	ND	0.01
Pesticides - Luke II Organochlorine	Endrin	ND	0.01
Pesticides - Luke II Organochlorine	Ethalfluralin	ND	0.01
Pesticides - Luke II Organochlorine	Dieldrin	ND	0.01
Pesticides - Luke II Organochlorine	Endosulfan, alpha-	ND	0.01
Pesticides - Luke II Organochlorine	Endosulfan beta	ND	0.01
Pesticides - Luke II Organochlorine	Dichlone	ND	0.05
Pesticides - Luke II Organochlorine	Dicloran	ND	0.01
Pesticides - Luke II Organochlorine	Dicofol	ND	0.02
Pesticides - Luke II Organochlorine	DDE	ND	0.01
Pesticides - Luke II Organochlorine	DDT	ND	0.01
Pesticides - Luke II Organochlorine	Dichlobenil	ND	0.01
Pesticides - Luke II Organochlorine	Cyanazine	ND	0.02
Pesticides - Luke II Organochlorine	Dacthal (Chlorthal)	ND	0.02
Pesticides - Luke II Organochlorine	DDD	ND	0.01
Pesticides - Luke II Organochlorine	Chlordane (total)	ND	0.01
Pesticides - Luke II Organochlorine	Chlorfenapyr	ND	0.01
Pesticides - Luke II Organochlorine	Chlorothalonil	ND	0.02
Pesticides - Luke II Organochlorine	Bromacil	ND	0.02
Pesticides - Luke II Organochlorine	Captafol	ND	0.01
Pesticides - Luke II Organochlorine	Captan	ND	0.02
Pesticides - Luke II Organochlorine	a, ß, d-BHC (Benzene hexachloride)	ND	0.01
Pesticides - Luke II Organochlorine	Bifenox	ND	0.03
Pesticides - Luke II Organochlorine	Boscalid	ND	0.02
Pesticides - Luke II Organochlorine	Alachlor	ND	0.02
Pesticides - Luke II Organochlorine	Aldrin	ND	0.01
Pesticides - Luke II Organochlorine	Benfluralin	ND	0.02
Pesticides - Luke II Organophosphorus	Acephate	ND	0.02
Pesticides - Luke II Organophosphorus	Azinphos-methyl	ND	0.02
Pesticides - Luke II Organophosphorus	Sulprofos	ND	0.02
Pesticides - Luke II Organonitrogen	Thiabendazole	ND	0.02
Pesticides - Luke II Carbamates	Oxamyl	ND	0.02
Pesticides - Luke II Carbamates	Propoxur	ND	0.02
Pesticides - Luke II Carbamates	Thiodicarb	ND	0.02
Pesticides - Luke II Carbamates	Methiocarb	ND	0.02
Pesticides - Luke II Carbamates	Methomyl	ND	0.02
Pesticides - Luke II Carbamates	o-Phenylphenol	ND	0.02
Pesticides - Luke II Carbamates	Carbaryl	ND	0.02
Pesticides - Luke II Carbamates	Carbofuran	ND	0.02

I. Conclusion

Method verification was performed on the listed samples using the Eurofins method KK149, *Steviol Glycosides (HPLC) (JECFA 2008, modified)* (JECFA, Joint FAO/WHO Expert Committee on Food Additives). KK149 is a validated method. The purpose of this study is to verify the performance of method KK149 on 5 lots of test material (samples) submitted and to confirm the composition and identification of said submitted test materials.

To verify method performance on these samples the following parameters were measured: single point calibration, selectivity, system suitability, accuracy, repeatability and precision. Criteria for each parameter were designed to meet or exceed industry standards (AOAC, USP, WHO).

Single point calibration procedures measured the performance of the analytical instrumentation and methodology in regards to standardization against reference material with a known purity at a specified sample concentration for improved accuracy and precision of measurement at that concentration. Based on the selected criteria for passing calibration, the method is acceptable for analysis and quantitation by KK149.

Selectivity for this study measured and confirmed that the analysis was free from interferences; as shown from the samples, and reagent blank test results. This study also confirmed that the method can determine the difference between closely related compounds, as shown in the separation of the two major peaks; stevioside and rebaudioside A in the positive control. Furthermore this study has proven that the method can properly identify compounds from the test material by retention time.

System suitability further confirms the performance of the method on test materials and standards and the equipments ability to respond consistently over time. Retention time indicates that the peak of interest is stable from sample to sample as the analysis progresses through time, where tailing factor and theoretical plate count describe the condition of the analytical column used for separation. When the criteria are met as it was in this study it indicates that the equipment is acceptable for quantitation and identification.

Accuracy is performed to indicate that the primary reference material in use is accurately portrayed by the manufacturer. In this instance the USP (primary reference material) accurately measured the value of the control sample indicating that the analysis is accurately measuring the concentration of rebaudioside A and related glycosides in submitted samples.

Repeatability and precision are performed to confirm the test material can be prepared and analyzed repeatedly with no statistically significant difference between generated results. This is not only a measure of the ability of the method to perform appropriately on multiple analyses but also assesses the homogeneity of the test material. On this study each test material was prepared in triplicate. To evaluate repeatability on this data the relative standard deviation (RSD) was

calculated. The RSD is calculated as the standard deviation divided by the average (of the measured values). All study RSDs passed the criteria. This result indicates the test material submitted (samples) were homogenous.

Results of this study further indicate that the KK149 is appropriate and verified for this test material.

Finally the analyses for residual solvents and pesticides show that the samples are effectively free from these components (below published specifications) or not detected by the methods of use.

Eurofins Scientific, Inc.
1365 Redwood Way
Petaluma, Ca 94951

Method Verification for the Determination of Rebaudioside A and Related Steviol Glycosides by High Performance Liquid Chromatography (HPLC) with Purity and Solvent Analysis of 5 Production Samples and Selected Pesticide Screening

(This report contains updates for clarification and supersedes the December 2010 report.)

Prepared by: (b) (6)
Jules Skamarack
Eurofins Scientific, Inc.

Reviewed by: (b) (6)
Mariel Esguerra
Eurofins Scientific, Inc.

Approved by: _____
Mathew Titlow
Chengdu Wagott Pharmaceutical Co., Ltd.

Date Issued: March 2011

(4) Rebaudioside A, Lot #(b) (4) , Eurofins sample number 740-2010-00007949

(5) Rebaudioside A, Lot #(b) (4) , Eurofins sample number 740-2010-00007950

6. Test Reagents:

(1) Acetonitrile, HPLC Grade
(2) Fisher P/N A998-4, VWR P/N JT9017-3
(3) Rebaudioside A Reference Material, Lot. F01077 from USP Catalogue # 1600121, C.A.S # 58543-16-1
(4) Positive control sample for day to day accuracy check identified as Eurofins control # LCKK149-2 monitored for rebaudioside A concentration.
(5) Phosphoric Acid, Fischer Chemical Company P/N A260
(6) Steviolbioside Reference Material, Lot # 19349-1821 from ChromaDex Catalogue # 19349010, CAS # 41093-60-1
(7) Rebaudioside B Reference Material, Lot # Lot # 18227-101 from ChromaDex Catalogue # ASB00018227, CAS # 58543-17-2
(8) Stevioside Reference Material, Lot # F01080 from USP Catalogue # 1622408, CAS # 471-80-7
(9) Rebaudioside C Reference Material, Lot # 00018228-3202 from ChromaDex Catalogue # ASB-00018228, CAS # 63550-99-2

Mobile Phase Preparation: (see attached method)

7. Method References:

High Performance Liquid Chromatographic Determination of Individual Sweet Diterpenoid Glycosides of *Stevia rebaudiana*, W.A.Court, Agriculture & Food Canada Pest Management Research Centre, P.O. Box 186, Ontario, N4B 2W9

Steviol glycosides, Prepared at the 69th JEFCA (2008) published in FAO JECFA Monographs 5 (2008) superseding specification prepared in the 68th JEFCA (2007), published in FAO JECFA Monographs 5 (2008). An ADI of 0-4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

II. Study Description

1. Scope:

This is applicable to the determination of rebaudioside A, stevioside and Stevia glycosides in 5 raw material samples.

2. Test Materials:

(1) Rebaudioside A, Lot #(b) (4) , Eurofins sample number 740-2010-00007946

(2) Rebaudioside A, Lot #(b) (4) , Eurofins sample number 740-2010-00007947

(3) Rebaudioside A, Lot #(b) (4) , Eurofins sample number 740-2010-00007948

(4) Rebaudioside A, Lot #(b) (4), Eurofins sample number 740-2010-00007949

(5) Rebaudioside A, Lot #(b) (4), Eurofins sample number 740-2010-00007950

3. Reference Standards: (rebaudioside A)

A. Stock standards:

1. The reference material was preparation for rebaudioside A by drying at 105 degrees Centigrade for two hours as directed by JECFA.

2. On a microbalance, accurately weigh 10.0 ± 1 mg of rebaudioside A USP reference material; quantitatively transfer to a 5-mL volumetric flask with mobile phase. Dissolve using heat if necessary. Cool to room temperature and dilute to volume with mobile phase. Concentration is approximately 2 mg/mL rebaudioside A.

3. Steviolbioside, rebaudioside B, stevioside and rebaudioside C reference materials were run as qualitative retention time markers. A sufficient amount of each material to create a detectable peak from the method was placed in an injection vial and solublized with mobile phase.

B. Reference standard preparation (USP rebaudioside A). A single point calibration is used per JECFA for determination of high purity samples. To accommodate this, the stock reference material is used as prepared. The actual concentration is adjusted for standard purity and is listed below for rebaudioside A:

Reference Standard Concentration (mg/ml)
2.0893578

4. Verification Study:

A. Primary method LC-KK149.00: (method is attached in **appendix #1**)

B. Single Point Calibration:

1. A single point calibration is used by injecting the reference stock standard a minimum of five times. This is a modification from the attached method used to achieve greater accuracy on high purity analyses.

a. Calculate response factors. RSD between injections must be ≤1.5%.

2. Results, rebaudioside A;

a. Response factors RSD between levels (amount) was found to be 0.0107614 and passed the criteria (appendix #2).

C. Selectivity: For purposes of this study, selectivity is specificity

1. Preparation solvent blank analysis:

a. Preparation solvent blanks are to be free of peaks.

ii. Result: Blanks were shown to be free of peaks. (file # 2010-10-18\1FA-0101.D and -0102D) (appendix # 3)

2. Positive control and accuracy sample analysis:

a. Analyze the positive control sample for the determination of accuracy and peak separation. The positive control is tested with each analytical run to verify concentration and proper dilution of the reference material into the reference standard for accuracy of the determination. The result of the positive control is monitored

using control charting. The measured concentration of rebaudioside A should be with-in 2 standard deviations of the mean. The mean result is established at 64.0 %(w/w) and the standard deviation is 0.281. This control was analyzed twice labeled as *control new* and *control old*, based upon where they stood in the analysis sequence.

ii. Result: Measured concentration results are 63.999018 and 64.473495 % (w/w) which passed the criteria. (file #'s 2010-10-18\1FE-0601.D and 2010-10-18\1BD-5001.D) (Appendix # 4)

3. Demonstrate separation of the two major peaks, stevioside and rebaudioside A in the positive control:

a. Separation was demonstrated with actual retention times of 3.976 minutes (stevioside) and 5.063 minutes (rebaudioside A) (file # 2010-10-18\1FE-0601.D) (Appendix # 4)

4. Retention time markers were tested to verify the retention time of the following related glycosides in both standards and samples; steviolbioside, rebaudioside B, Stevioside and rebaudioside C. **(Appendix # 5)**

D. System Suitability:

1. The reference stock standard 2.089357 mg/ml rebaudioside A solution is injected after every five to six sample injections and at the end of the analysis sequence.

a. Acceptance criteria: The system is considered suitable if the retention times of the standard peaks do not deviate more than 0.5 minutes and the RSD of the peak areas are less than 2%.

ii. Results: Average retention time 5.066 minutes with a RSD of 0.095. The actual deviation between the longest and shortest retention time is 0.019 (5.077 minutes to 5.058 minutes) minutes. All criteria pass. (Appendix # 6)

2. USP tailing factor was determined for the rebaudioside A peak from the sample matrix. The tailing factor should be not more than (NMT) 2.0. The tailing factors from the run labeled A of each sample are listed below:

Sample	USP Tailing Factor
7946A	0.958
7947A	0.958
7948A	0.956
7949A	0.959
7950A	0.955

a. Results: The tailing factor passed the criteria (USP) at 0.957 (average). (Appendix # 7)

3. Column Efficiency, Not less than (NLT) 5000 theoretical plate count, using the Rebaudioside A peak from the sample matrix.

Sample	Plates (Halfwidth method)
7946A	7268
7947A	7491
7948A	7491
7949A	7484
7950A	7242

a. Results: All theoretical plate count calculations were greater than 5000 with the halfwidth **method calculating the average of all samples at 7395. All pass the criteria. (Appendix # 7)**

4. USP tailing factor was determined for the rebaudioside A standard peak at the 2.0934276 mg/ml concentration and was determined on 12 injections. The tailing factor should be not more than (NMT) 2.0.

a. Results: The tailing factor passed the criteria (USP) at 0.954. (Appendix # 8)

5. Column efficiency, Not less than (NLT) 5000 theoretical plate count, using the rebaudioside A standard peak at the 2.0934276 mg/ml concentration and was determined on 12 injections.

a. Results: All theoretical plate count calculations were greater than 5000 with the halfwith method calculating at 7356 (average). All pass the criteria. (Appendix # 8)

E. Accuracy:

1. Accuracy was examined in the initial validation for the method KK149. For purposes of this study, the USP reference standard was compared to the Eurofins control sample LCKK149-2. Accuracy based on the analysis of the control sample passes the criteria. See section 4C2 and **appendix 4.** Additionally, Section F (Accuracy) from the LC-KK149.00 method validation report is included with example chromatograms **(Appendix # 9)**

F. Repeatability and Precision:

1. For each sample, perform 3 replicate sample preparations. The sample assay preparation concentration was diluted to approximately 2 mg/ml to match the concentration of the rebaudioside reference standard and was based on the expected test sample concentration. All samples were dried to the anhydrous solvent free basis at 105 degrees Centigrade for 2 hours as directed by JECFA. The acceptance criteria for the relative standard deviation (RSD) for each set of 3 analyses must be $\leq$ 5% for the total steviol glycoside value.

a. Results: As seen in the following table, RSDs for rebaudioside A on all samples pass the criteria. In addition all measurements confirmed a minimum passing specification for rebaudioside A of 95% dry weight, solvent free basis. The retention time of the peak rebaudioside A in the chromatogram of the assay preparation corresponds to that in the chromatogram of the reference standard preparation as obtained during the Assay confirming the identification of the material. Test material sample chromatograms are located in appendix # 10.

Sample 7946	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.034601	0.036221	0.039898	0.0369067	7.354364
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.2809	1.2849	1.2729	1.2795667	0.4775133
Stevioside	0.97177	0.94766	0.95604	0.95849	1.2770397
Rebaudioside C	0.76694	0.77745	0.77369	0.7726933	0.6892015
Unknown	0.60265	0.61755	0.6269	0.6157	1.9864207
Rebaudioside A	95.017	95.019	95.041	95.025667	**0.0140137**
Total	98.674	98.682	98.711	98.689	0.0197265

Sample 7947	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.030008	0.0370072	0.034937	0.0339841	10.580221
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.3065	1.2986	1.3062	1.3037667	0.343388
Stevioside	1.0763	1.0699	1.0965	1.0809	1.284467
Rebaudioside C	0.81024	0.82954	0.82565	0.82181	1.2420077
Unknown	0.63254	0.62296	0.65837	0.6379567	2.8710259
Rebaudioside A	97.977	97.719	97.603	97.766333	**0.195814**
Total	101.82	101.58	101.52	101.64	0.1561837

Sample 7948	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.034792	0.036266	0.035551	0.0355363	2.0742417
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.2956	1.3111	1.2726	1.2931	1.4980567
Stevioside	1.0011	1.0308	1.0009	1.0109333	1.7019251
Rebaudioside C	0.89892	0.91213	0.9019	0.9043167	0.7661755
Unknown	0.71476	0.77668	0.71965	0.73703	4.6707521
Rebaudioside A	97.505	97.096	97.014	97.205	**0.2705857**
Total	101.45	101.16	100.49	101.03333	0.4873394
Sample 7949	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.035686	0.030632	0.032974	0.0330973	7.6418733
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.4687	1.4678	1.5188	1.4851	1.9654249
Stevioside	1.0309	1.0364	1.0861	1.0511333	2.8927475
Rebaudioside C	0.90192	0.93049	0.97317	0.9351933	3.8341912
Unknown	0.74674	0.70001	0.78405	0.7436	5.6627082
Rebaudioside A	97.533	97.104	97.090	97.242333	**0.2589634**
Total	101.72	101.27	101.49	101.49333	0.2217077

Sample 7950	Run 1	Run 2	Run 3		
Compound	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Result (%w/w) moisture corrected	Average	Relative Standard Deviation
Steviobioside	0.039603	0.035764	0.03232	0.035046	10.395722
Dulcoside A	<0.01	<0.01	<0.01	n/a	n/a
Rebaudioside B	1.5119	1.4648	1.4699	1.4822	1.7438301
Stevioside	1.2482	1.2486	1.2437	1.2468333	0.2182254
Rebaudioside C	0.90182	0.96317	0.97761	0.9475333	4.2470162
Unknown	0.75445	0.74629	0.75924	0.7533267	0.8691679
Rebaudioside A	97.437	97.232	97.101	97.256667	**0.1741296**
Total	101.89	101.69	101.58	101.72	0.1545049

G. Analysis of residual solvents for ethanol and methanol was performed following USP <467> criteria. Results are listed below with the accompanying USP and JEFCA specification for these residuals. **All samples pass the criteria.**

	Result (mg/kg)		JECFA Specification (mg/kg)		USP/FCC Specification (mg/kg)	
Sample	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
7946	<50	90	NMT 200	NMT 5000	NMT 200	NMT 5000
7947	<50	270	NMT 200	NMT 5000	NMT 200	NMT 5000
7948	82	67	NMT 200	NMT 5000	NMT 200	NMT 5000
7949	<50	78	NMT 200	NMT 5000	NMT 200	NMT 5000
7950	<50	84	NMT 200	NMT 5000	NMT 200	NMT 5000

H. Analysis of pesticide residuals was also performed following the criteria listed above. The samples were found to be free of pesticides (none detected ND) at the listed limits of quantitation (LOQ) in the table below.

Sample 7946 Pesticide Screen FDA PAM 302 E7C6 (mg/kg)			
Screen	Pesticide	Result	LOQ
Pesticides - Luke II Carbamates	Oxamyl	ND	0.02
Pesticides - Luke II Carbamates	Propoxur	ND	0.02
Pesticides - Luke II Carbamates	Thiodicarb	ND	0.02
Pesticides - Luke II Carbamates	Methiocarb	ND	0.02
Pesticides - Luke II Carbamates	Methomyl	ND	0.02
Pesticides - Luke II Carbamates	o-Phenylphenol	ND	0.02
Pesticides - Luke II Carbamates	Carbaryl	ND	0.02
Pesticides - Luke II Carbamates	Carbofuran	ND	0.02
Pesticides - Luke II Carbamates	Carbofuran 3-OH	ND	0.02
Pesticides - Luke II Carbamates	Aldicarb	ND	0.02
Pesticides - Luke II Carbamates	Aldicarb sulfone	ND	0.02
Pesticides - Luke II Carbamates	Aldicarb sulfoxide	ND	0.02
Pesticides - Luke II Organophosphorus	Thionazin	ND	0.02
Pesticides - Luke II Organophosphorus	Propetamphos	ND	0.02
Pesticides - Luke II Organophosphorus	Ronnel	ND	0.02
Pesticides - Luke II Organophosphorus	Tetrachlorvinphos	ND	0.02
Pesticides - Luke II Organophosphorus	Phosphamidon	ND	0.02
Pesticides - Luke II Organophosphorus	Pirimiphos-methyl	ND	0.02
Pesticides - Luke II Organophosphorus	Profenofos	ND	0.02
Pesticides - Luke II Organophosphorus	Phorate	ND	0.02
Pesticides - Luke II Organophosphorus	Phosalone	ND	0.02
Pesticides - Luke II Organophosphorus	Phosmet	ND	0.02
Pesticides - Luke II Organophosphorus	Mevinphos	ND	0.02
Pesticides - Luke II Organophosphorus	Omethoate	ND	0.02
Pesticides - Luke II Organophosphorus	Parathion	ND	0.02
Pesticides - Luke II Organophosphorus	Methamidophos	ND	0.02
Pesticides - Luke II Organophosphorus	Methidathion	ND	0.02

Pesticides - Luke II Organophosphorus	Methyl Parathion	ND	0.02
Pesticides - Luke II Organophosphorus	Isofenphos	ND	0.02
Pesticides - Luke II Organophosphorus	Malathion	ND	0.02
Pesticides - Luke II Organophosphorus	Metasystox-R	ND	0.02
Pesticides - Luke II Organophosphorus	Fenitrothion	ND	0.02
Pesticides - Luke II Organophosphorus	Fenthion	ND	0.02
Pesticides - Luke II Organophosphorus	Fonofos	ND	0.02
Pesticides - Luke II Organophosphorus	Ethion	ND	0.02
Pesticides - Luke II Organophosphorus	Ethoprop	ND	0.02
Pesticides - Luke II Organophosphorus	Fenamiphos	ND	0.02
Pesticides - Luke II Organophosphorus	Dimethoate	ND	0.02
Pesticides - Luke II Organophosphorus	Disulfoton	ND	0.02
Pesticides - Luke II Organophosphorus	EPN	ND	0.02
Pesticides - Luke II Organophosphorus	Diazinon	ND	0.02
Pesticides - Luke II Organophosphorus	Dibrom (Naled)	ND	0.02
Pesticides - Luke II Organophosphorus	Dicrotophos	ND	0.02
Pesticides - Luke II Organophosphorus	Coumaphos	ND	0.02
Pesticides - Luke II Organophosphorus	DEF	ND	0.02
Pesticides - Luke II Organophosphorus	Demeton group	ND	0.02
Pesticides - Luke II Organophosphorus	Chlorpyrifos	ND	0.02
Pesticides - Luke II Organophosphorus	Chlorpyrifos-methyl	ND	0.02
Pesticides - Luke II Organophosphorus	Ciodrin (Crotoxyphos)	ND	0.02
Pesticides - Luke II Organophosphorus	Bensulide	ND	0.02
Pesticides - Luke II Organophosphorus	Carbophenothion	ND	0.02
Pesticides - Luke II Organophosphorus	Chlorfenvinphos	ND	0.02
Pesticides - Luke II Organonitrogen	Simazine	ND	0.02
Pesticides - Luke II Organonitrogen	Tebuconazole	ND	0.02
Pesticides - Luke II Organonitrogen	Terbacil	ND	0.02
Pesticides - Luke II Organonitrogen	Pymetrozine	ND	0.02
Pesticides - Luke II Organonitrogen	Pyraclostrobin	ND	0.02
Pesticides - Luke II Organonitrogen	Pyriproxyfen	ND	0.02
Pesticides - Luke II Organonitrogen	Prometon	ND	0.02
Pesticides - Luke II Organonitrogen	Prometryn	ND	0.02
Pesticides - Luke II Organonitrogen	Propamocarb	ND	0.02
Pesticides - Luke II Organonitrogen	Metalaxyl	ND	0.02
Pesticides - Luke II Organonitrogen	Metolachlor	ND	0.02
Pesticides - Luke II Organonitrogen	Molinate	ND	0.02
Pesticides - Luke II Organonitrogen	Hexazinone	ND	0.02
Pesticides - Luke II Organonitrogen	Imazalil	ND	0.02
Pesticides - Luke II Organonitrogen	Kresoxim-methyl	ND	0.02
Pesticides - Luke II Organonitrogen	Fenamidone	ND	0.02
Pesticides - Luke II Organonitrogen	Fipronil	ND	0.02
Pesticides - Luke II Organonitrogen	Fludioxonil	ND	0.02
Pesticides - Luke II Organonitrogen	Cyprodinil	ND	0.02
Pesticides - Luke II Organonitrogen	Dimethomorph	ND	0.02
Pesticides - Luke II Organonitrogen	Diphenylamine	ND	0.02
Pesticides - Luke II Organonitrogen	Benthiocarb	ND	0.02
Pesticides - Luke II Organonitrogen	Cyanazine	ND	0.02
Pesticides - Luke II Organonitrogen	Cyromazine	ND	0.02
Pesticides - Luke II Organonitrogen	Acetamiprid	ND	0.02
Pesticides - Luke II Organonitrogen	Atrazine	ND	0.02
Pesticides - Luke II Organonitrogen	Azoxystrobin	ND	0.02
Pesticides - Luke II Pyrethroids	Tralomethrin	ND	0.02
Pesticides - Luke II Pyrethroids	Fluvalinate	ND	0.02
Pesticides - Luke II Pyrethroids	Cyhalothrin lambda	ND	0.02
Pesticides - Luke II Pyrethroids	Permethrin	ND	0.02
Pesticides - Luke II Pyrethroids	Deltamethrin	ND	0.02
Pesticides - Luke II Pyrethroids	Fenpropathrin	ND	0.02
Pesticides - Luke II Pyrethroids	Esfenvalerate	ND	0.02
Pesticides - Luke II Pyrethroids	Bifenthrin	ND	0.02
Pesticides - Luke II Pyrethroids	Cyfluthrin	ND	0.02
Pesticides - Luke II Pyrethroids	Cypermethrin	ND	0.02
Pesticides - Luke II Organochlorine	Pyrethrins (sum of 6)	ND	0.02
Pesticides - Luke II Organochlorine	HCB (Hexachlorbenzene)	ND	0.01
Pesticides - Luke II Organochlorine	Metribuzin	ND	0.01
Pesticides - Luke II Organochlorine	Myclobutanil	ND	0.01
Pesticides - Luke II Organochlorine	Vegadex	ND	0.02
Pesticides - Luke II Organochlorine	Vinclozolin	ND	0.02
Pesticides - Luke II Organochlorine	Chlorobenzilate	ND	0.04
Pesticides - Luke II Organochlorine	Trifloxystrobin	ND	0.01

Pesticides - Luke II Organochlorine	Triflumizole	ND	0.01
Pesticides - Luke II Organochlorine	Trifluralin	ND	0.02
Pesticides - Luke II Organochlorine	Tetradifon	ND	0.02
Pesticides - Luke II Organochlorine	Toxaphene (camphechlor)	ND	0.01
Pesticides - Luke II Organochlorine	Tridimephon	ND	0.02
Pesticides - Luke II Organochlorine	Profluralin	ND	0.02
Pesticides - Luke II Organochlorine	Pronamide	ND	0.02
Pesticides - Luke II Organochlorine	Propanil	ND	0.01
Pesticides - Luke II Organochlorine	Perthane	ND	0.01
Pesticides - Luke II Organochlorine	Polychlorinated Biphenyls	ND	0.01
Pesticides - Luke II Organochlorine	Procymidone	ND	0.01
Pesticides - Luke II Organochlorine	Pendimethalin	ND	0.01
Pesticides - Luke II Organochlorine	Pentachloronitrobenzene (PCNB)	ND	0.01
Pesticides - Luke II Organochlorine	Pentachloroaniline	ND	0.01
Pesticides - Luke II Organochlorine	Mirex	ND	0.01
Pesticides - Luke II Organochlorine	Oxadiazon	ND	0.02
Pesticides - Luke II Organochlorine	Oxyfluorfen	ND	0.01
Pesticides - Luke II Organochlorine	Lindane (gamma-HCH)	ND	0.01
Pesticides - Luke II Organochlorine	Linuron	ND	0.1
Pesticides - Luke II Organochlorine	Methoxychlor	ND	0.02
Pesticides - Luke II Organochlorine	Heptachlor epoxide	ND	0.01
Pesticides - Luke II Organochlorine	Indoxacarb	ND	0.02
Pesticides - Luke II Organochlorine	Iprodione	ND	0.02
Pesticides - Luke II Organochlorine	Fenhexamid	ND	0.02
Pesticides - Luke II Organochlorine	Folpet	ND	0.02
Pesticides - Luke II Organochlorine	Heptachlor	ND	0.01
Pesticides - Luke II Organochlorine	Endosulfan-sulfate	ND	0.01
Pesticides - Luke II Organochlorine	Endrin	ND	0.01
Pesticides - Luke II Organochlorine	Ethalfluralin	ND	0.01
Pesticides - Luke II Organochlorine	Dieldrin	ND	0.01
Pesticides - Luke II Organochlorine	Endosulfan, alpha-	ND	0.01
Pesticides - Luke II Organochlorine	Endosulfan beta	ND	0.01
Pesticides - Luke II Organochlorine	Dichlone	ND	0.05
Pesticides - Luke II Organochlorine	Dicloran	ND	0.01
Pesticides - Luke II Organochlorine	Dicofol	ND	0.02
Pesticides - Luke II Organochlorine	DDE	ND	0.01
Pesticides - Luke II Organochlorine	DDT	ND	0.01
Pesticides - Luke II Organochlorine	Dichlobenil	ND	0.01
Pesticides - Luke II Organochlorine	Cyanazine	ND	0.02
Pesticides - Luke II Organochlorine	Dacthal (Chlorthal)	ND	0.02
Pesticides - Luke II Organochlorine	DDD	ND	0.01
Pesticides - Luke II Organochlorine	Chlordane (total)	ND	0.01
Pesticides - Luke II Organochlorine	Chlorfenapyr	ND	0.01
Pesticides - Luke II Organochlorine	Chlorothalonil	ND	0.02
Pesticides - Luke II Organochlorine	Bromacil	ND	0.02
Pesticides - Luke II Organochlorine	Captafol	ND	0.01
Pesticides - Luke II Organochlorine	Captan	ND	0.02
Pesticides - Luke II Organochlorine	a, ß, d-BHC (Benzene hexachloride)	ND	0.01
Pesticides - Luke II Organochlorine	Bifenox	ND	0.03
Pesticides - Luke II Organochlorine	Boscalid	ND	0.02
Pesticides - Luke II Organochlorine	Alachlor	ND	0.02
Pesticides - Luke II Organochlorine	Aldrin	ND	0.01
Pesticides - Luke II Organochlorine	Benfluralin	ND	0.02
Pesticides - Luke II Organophosphorus	Acephate	ND	0.02
Pesticides - Luke II Organophosphorus	Azinphos-methyl	ND	0.02
Pesticides - Luke II Organophosphorus	Sulprofos	ND	0.02
Pesticides - Luke II Organonitrogen	Thiabendazole	ND	0.02
Pesticides - Luke II Carbamates	Oxamyl	ND	0.02
Pesticides - Luke II Carbamates	Propoxur	ND	0.02
Pesticides - Luke II Carbamates	Thiodicarb	ND	0.02
Pesticides - Luke II Carbamates	Methiocarb	ND	0.02
Pesticides - Luke II Carbamates	Methomyl	ND	0.02
Pesticides - Luke II Carbamates	o-Phenylphenol	ND	0.02
Pesticides - Luke II Carbamates	Carbaryl	ND	0.02
Pesticides - Luke II Carbamates	Carbofuran	ND	0.02

I. Conclusion

Method verification was performed on the listed samples using the Eurofins method KK149, *Steviol Glycosides (HPLC) (JECFA 2008, modified)* (JECFA, Joint FAO/WHO Expert Committee on Food Additives). KK149 is a validated method. The purpose of this study is to verify the performance of method KK149 on 5 lots of test material (samples) submitted and to confirm the composition and identification of said submitted test materials.

To verify method performance on these samples the following parameters were measured: single point calibration, selectivity, system suitability, accuracy, repeatability and precision. Criteria for each parameter were designed to meet or exceed industry standards (AOAC, USP, WHO).

Single point calibration procedures measured the performance of the analytical instrumentation and methodology in regards to standardization against reference material with a known purity at a specified sample concentration for improved accuracy and precision of measurement at that concentration. Based on the selected criteria for passing calibration, the method is acceptable for analysis and quantitation by KK149.

Selectivity for this study measured and confirmed that the analysis was free from interferences; as shown from the samples, and reagent blank test results. This study also confirmed that the method can determine the difference between closely related compounds, as shown in the separation of the two major peaks; stevioside and rebaudioside A in the positive control. Furthermore this study has proven that the method can properly identify compounds from the test material by retention time.

System suitability further confirms the performance of the method on test materials and standards and the equipments ability to respond consistently over time. Retention time indicates that the peak of interest is stable from sample to sample as the analysis progresses through time, where tailing factor and theoretical plate count describe the condition of the analytical column used for separation. When the criteria are met as it was in this study it indicates that the equipment is acceptable for quantitation and identification.

Accuracy is performed to indicate that the primary reference material in use is accurately portrayed by the manufacturer. In this instance the USP (primary reference material) accurately measured the value of the control sample indicating that the analysis is accurately measuring the concentration of rebaudioside A and related glycosides in submitted samples.

Repeatability and precision are performed to confirm the test material can be prepared and analyzed repeatedly with no statistically significant difference between generated results. This is not only a measure of the ability of the method to perform appropriately on multiple analyses but also assesses the homogeneity of the test material. On this study each test material was prepared in triplicate. To evaluate repeatability on this data the relative standard deviation (RSD) was

calculated. The RSD is calculated as the standard deviation divided by the average (of the measured values). All study RSDs passed the criteria. This result indicates the test material submitted (samples) were homogenous.

Results of this study further indicate that the KK149 is appropriate and verified for this test material.

Finally the analyses for residual solvents and pesticides show that the samples are effectively free from these components (below published specifications) or not detected by the methods of use.

APPENDIX C-2 PART 2

Appendix 1 through 11 Providing Details of:

1. **Analytical Method**
2. **Calibration**
3. **Selectivity (Solvent Blank)**
4. **Accuracy/Selectivity (Positive Control)**
5. **Retention Time Markers**
6. **Reference Standard System Suitability**
7. **Sample Suitability**
8. **Reference Standards System Suitability (USP Tailing Factor, Theoretical Plates)**
9. **Accuracy**
10. **Repeatability, Precision, Results (Sample Chromatogram)**
11. **Rebaudioside D & F Chromatograms**

Appendix #1

Analytical Method

eurofins

METHOD

Title	Number	Effective Date
Determination of Rebaudioside A and Related Steviol Glycosides by HPLC – modified JECFA KK149	**LC-KK149.00**	**12/11/2009**

Purpose

This method is for the determination of Rebaudioside A (Reb A), Stevioside, Dulcoside A, Steviolbioside, Rebaudioside C (Reb C), and Rebaudioside B (Reb B) by high performance liquid chromatography (HPLC). Quantitation of Stevioside, Dulcoside A, Steviolbioside, Reb C, and Reb B are based on the response of Reb A standard using the JECFA established conversion factors. The method is applicable to raw materials (purities) and finished products.

Definitions

N/A

Responsibility

Senior Operations will implement this method. Only properly trained personnel may perform this method. The revision of or any deviation from this method requires notification and approval of supervisory personnel.

Safety

Observe all standard laboratory safety procedures.

Environmental Conditions

N/A

Equipment

HPLC, Agilent 1100 HPLC or equivalent
Column: Zorbax NH_2, 4.6 x 150mm, 5 micron, or equivalent
Analytical balance, 0.0001 g resolution
Microbalance, 0.00001 g resolution
pH Meter
Sonicator
Serological pipets
Class A pipettes, various sizes
Disposable glass pipets, various sizes
0.45-µm PTFE filter
Graduated cylinder, 1000-mL
Glass eluent bottles, 1000-mL

Approved by:

Quality Manager	Date

VOA vials, 20-mL and 40-mL
Amber autosampler vials
Disposable syringes, 5-mL

Reference Materials/Reagents

Rebaudioside A (Reb A) standard, USP Reference Standard
Phosphoric acid(H_3PO_4), HPLC grade
Acetonitrile, HPLC Grade
Milli-Q water, fresh daily

Quality Control Plan

1. A preparation solvent blank must be free of interfering peaks, and is analyzed every ten samples.
2. Linearity must be demonstrated by a 3-point calibration standard or other means. Correlation coefficients of standard curves must be greater than 0.999.
3. The %RSD of a minimum of five replicate injections of the 1mg/ml standard must not be greater than 1.5.
4. Response factors of standard calibration levels must agree within 10% of the average of the response factors for the complete calibration curve.
5. Bracket each run with standard injections, and include an additional standard injection after every five sample injections.
6. Every tenth sample in a set must be prepared and analyzed in duplicate. If the set is fewer than ten samples, one sample in the set must be run in duplicate. The percent difference between duplicate results must be less than ten for finished products and less than two for purity samples.
7. A laboratory control sample of known concentration of Reb A, is to be analyzed with each run. Control charting is performed according to SOP QUA-003, Quality Control, Control Charting and Proficiency Testing.
8. If expected levels or specifications have been provided, the sample area count must fall within the area counts of the standard curve.
9. Beer's Law must be met.

Procedure

Mobile Phase/Preparation Solvent Preparation:

1. Using a graduated cylinder, measure 800 mL of acetonitrile and transfer to a fresh 1000-mL glass eluent bottle.
2. Transfer 200 mL of milli-Q water, via a graduated cylinder to the eluent bottle.
3. Swirl to mix and label appropriately.
4. Using a pH meter, adjust the pH to 3.0 ± 0.05 with phosphoric acid.

Standard Preparation:

1. Using a commercially available standard, on a microbalance, accurately weigh approximately 10 mg of Reb A and transfer to a 20-ml VOA vial.

NOTE: If using a premixed liquid Rebaudioside A standard, perform the necessary dilutions to obtain a minimum 3-point curve.

2. Using a 5-ml class A pipet, transfer 5 ml of preparation solvent to the vial.
3. Sonicate to dissolve.
4. Prepare dilutions of the stock solution to create a minimum 3-point calibration curve. Recommended dilutions: 1:2 (approximately 1mg/mL) and 1:5.
5. Transfer the standard solutions to amber HPLC autosampler vials and cap.

Note: Correct the standard for purity and moisture using the following calculations:

Purity:

$$[\text{standard}_{mg/mL}]_{corrected} = \frac{[\text{standard}_{mg/mL}] \times \%\ \text{purity}}{100\%}$$

Moisture:

$$[\text{standard}_{mg/mL}] = \frac{(100\% - \%\ \text{moisture})}{100\%} \times [\text{standard}_{mg/mL}]_{corrected}$$

Sample Preparation:

1. For raw materials, dry sample at 105°C for 2 hours to obtain moisture content. Use the dried material for analysis.
2. Sample size should be based on label claim or estimates and prepared according to the calibration standard levels. Weigh an accurate amount of sample on an analytical balance into a 40-mL VOA vial. For a typical reb A raw material (~97% powder) purity determination, weigh approximately 40 mg into a 40-mL VOA vial.
3. Using a class A volumetric pipet, dilute the sample with 40 mL of preparation solvent.
4. Sonicate for 30 ± 5 minutes.
5. If warming due to sonication has occurred, allow the sample to come to room temperature.
6. Filter through a 0.45µm PTFE syringe filter into an appropriately labeled amber autosampler vial, cap and analyze.

Instrument Conditions

Column Temperature: 35°C
Detection: UV 210 nm
Flow Rate: 1.0 mL/minute
Injection Volume: 5.0 µL
Gradient Program:

Time (minutes)	Mobile Phase (% ratio)	Milli Q Water (% ratio)
0	100	0
16	100	0
17	70	30
20	70	30
21	100	0

Title: Determination of Rebaudioside A and related steviol glycosides by HPLC – KK149
Number: LC-KK149.00

	24	100	0
Post Time:	10 minutes		

Retention Times: (Approximate retention times):

steviolbioside	~5.9 mins
dulcoside A	~7.2 mins
rebaudioside B	~7.9 mins
stevioside	~8.8 mins
rebaudioside C	~9.6 mins
unknown	~10.0 mins
rebaudioside A	~11.7 mins

Calculations

Determine the peak areas of Rebaudioside A in the standard chromatograms. Quantitate the other major steviol glycosides based on the average response of the Rebaudioside A standard. The concentrations of the following steviol glycosides are calculated using the following conversion factors:

Compound	MW	Conversion Reb A to listed compound
rebaudioside A	967.03	1.00
stevioside	804.874	0.83
unknown	n/a	1.00
dulcoside A	788.87	0.82
steviolbioside	642.73	0.66
rebaudioside C	951.02	0.98
rebaudioside B	804.874	0.83

$$\% \text{ glycoside} = \frac{\text{Area (sample)} \times [\text{standard}] \times 100}{\text{Area (standard)} \times [\text{sample}]}$$

where,

[ ] standard/sample is in mg/mL

References

Steviol glycosides, Prepared at the 69th JECFA (2008) published in FAO JECFA Monographs 5 (2008) superseding specification prepared in the 68th JECFA (2007), published in FAO JECFA Monographs 5 (2008). An ADI of 0-4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008)

Revision History

Title: Determination of Rebaudioside A and related steviol glycosides by HPLC – KK149
Number: LC-KK149.00

Effective Date	Version	Pages Affected	Reason/Summary of Changes
12/11/2009	00	all	New

Appendix #2

Calibration

Statistic Report

Sequence table: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA-7941.S
Data directory path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Ver: 1
Operator: Mariel Esguerra

Method file name: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M

Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	P1-F-02	1	10/18/2010 7:58:43 PM	1FB-0201.D	Rebaudioside Stk
2	P1-F-02	1	10/18/2010 8:38:25 PM	1FB-0301.D	Rebaudioside Stk
3	P1-F-02	2	10/18/2010 9:18:09 PM	1FB-0302.D	Rebaudioside Stk
4	P1-F-02	3	10/18/2010 9:57:52 PM	1FB-0303.D	Rebaudioside Stk
5	P1-F-02	4	10/18/2010 10:37:36 PM	1FB-0304.D	Rebaudioside Stk

Statistic results for compound Steviolbioside not available.

Statistic results for compound Rebaudioside B not available.

Statistic results for compound Dulcoside A not available.

Statistic results for compound Stevioside not available.

Statistic results for compound Rebaudioside C not available.

Compound: Rebaudioside A (Signal: DAD1 A, Sig=210,4 Ref=off)

Run #	Type	RetTime [min]	Amount [mg/mL]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	MM	5.053	2.07332	2362.30298	252.74579	0.1558	1.08
2	MF	5.059	2.07329	2362.25586	252.26880	0.1561	1.08
3	MM	5.060	2.07363	2362.69458	253.83768	0.1551	1.08
4	MM	5.062	2.07343	2362.46753	253.27986	0.1555	1.08
5	MM	5.062	2.07302	2361.97021	253.41577	0.1553	1.08
Mean:		5.059	2.07334	2362.33823	253.10958	0.1556	1.08
S.D.:		3.77e-3	2.23119e-4	2.67940e-1	6.10764e-1	3.69e-4	3e-3
RSD :		0.074	1.07614e-2	1.13421e-2	2.41304e-1	0.2373	0.29
95% CI:		4.68e-3	2.77039e-4	3.32691e-1	7.58364e-1	4.58e-4	4e-3

Statistic results for compound Unknown not available.

S t a t i s t i c R e p o r t

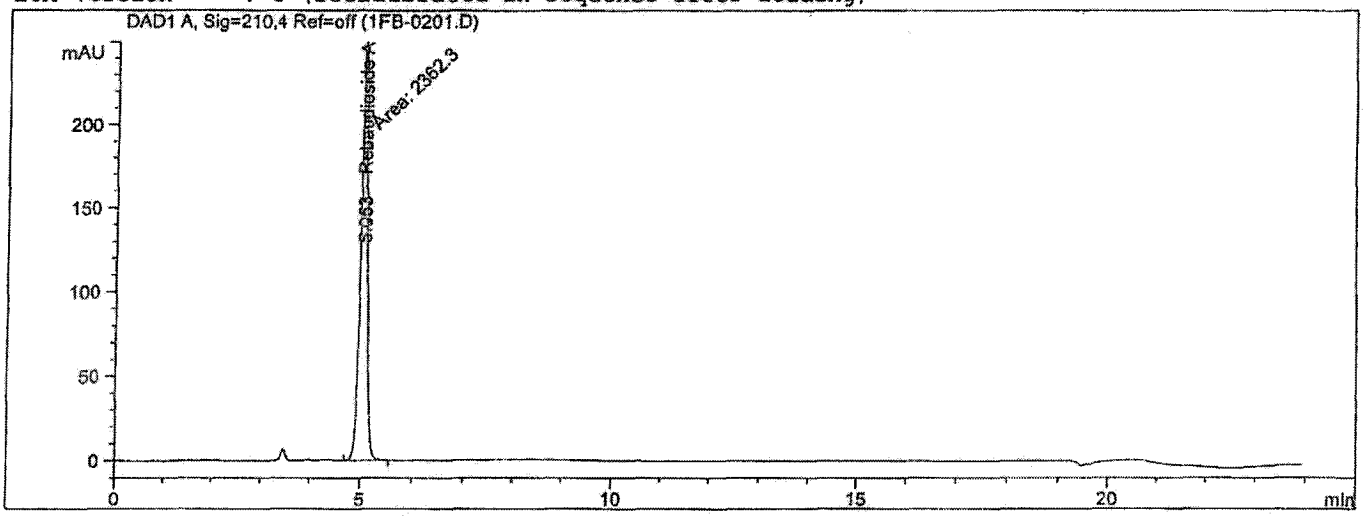
Sequence table: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA-7941.S
Data directory path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Ver: 1
Operator: Mariel Esguerra

No (or not enough) sample runs with cal. compounds available!

Sample Name: Rebaudioside Stk

```
Acq. Operator    : Mariel Esguerra                  Seq. Line :   2
Acq. Instrument  : HPLC 10                           Location : P1-F-02
Injection Date   : 10/18/2010 7:58:43 PM                 Inj :   1
                                                  Inj Volume : 5.0 µl
Sequence File    : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA-7941.S
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 12:03:24 PM by Mariel Esguerra
                   (recalibrated in sequence after loading)
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1 (recalibrated in sequence after loading)
```



Calibration Table (after recalibration)

Calib. Data Modified : Wednesday, October 20, 2010 12:03:24 PM

Level 1 calibrated: Replace Response Factors,
Replace Retention Times

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Sig	Lvl	Amount [mg/mL]	Area	Amt/Area	Ref	Grp	Name
3.006	1	1	0.00000	1.00000	0.00000			Steviolbioside
3.852	1	1	0.00000	1.00000	0.00000			Rebaudioside B
4.143	1	1	0.00000	1.00000	0.00000			Dulcoside A
4.466	1	1	0.00000	1.00000	0.00000			Stevioside
4.918	1	1	0.00000	1.00000	0.00000			Rebaudioside C
5.053	1	3	4.17872e-1	478.74072	8.72856e-4			Rebaudioside A
		2	1.04468	1192.86121	8.75776e-4			
		1	2.08936	2362.30298	8.84458e-4			
5.800	1	1	0.00000	1.00000	0.00000			Unknown

000100

Sample Name: Rebaudioside Stk

Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min
Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Ret. Times	:	No, only for identified peaks
Curve Type	:	Average Response/Amount
Origin	:	Ignored
Weight	:	Equal

1 Warnings or Errors :

Warning : Overlapping peak time windows at 4.918 min, signal 1

==========

External Standard Report (Sample Amount is 0!) (after recalibration)

==========

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:03:24 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.053	MM	2362.30298	8.77669e-4	2.07332		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07332		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

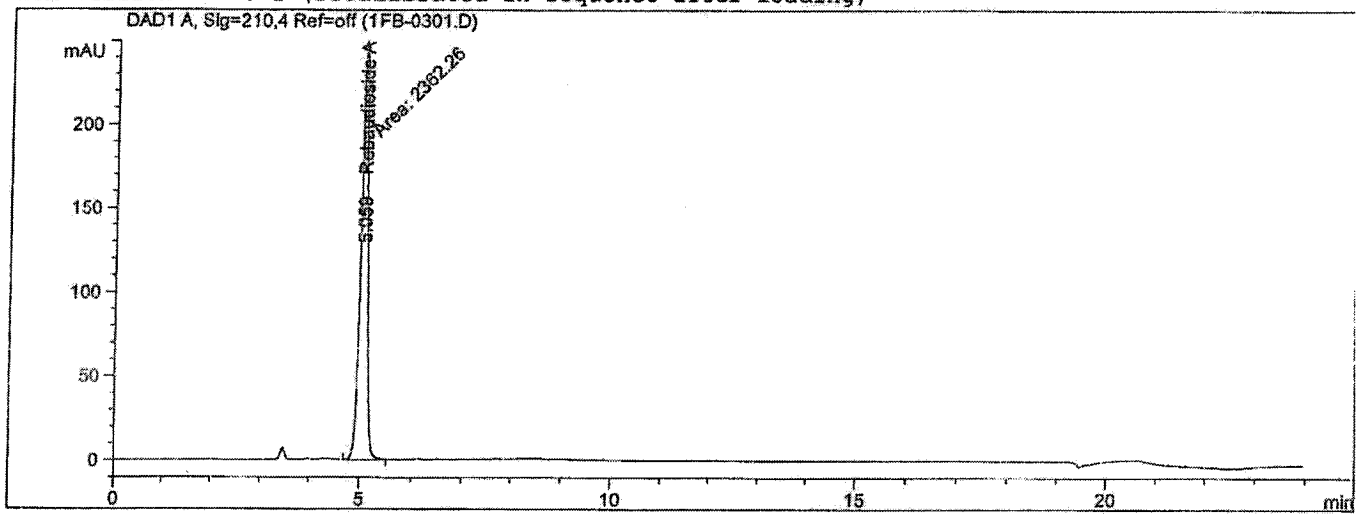
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

Acq. Operator	: Mariel Esguerra	Seq. Line	: 3
Acq. Instrument	: HPLC 10	Location	: P1-F-02
Injection Date	: 10/18/2010 8:38:25 PM	Inj	: 1
		Inj Volume	: 5.0 µl
Sequence File	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA-7941.S	
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA.M	
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA.M	
Last changed	: 10/20/2010 12:03:29 PM by Mariel Esguerra (recalibrated in sequence after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (recalibrated in sequence after loading)



Calibration Table (after recalibration)

Calib. Data Modified : Wednesday, October 20, 2010 12:03:29 PM

Level 1 calibrated: Average Response Factors of all calibrations, Floating Average of Retention Times, New 75%

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Sig	Lvl	Amount [mg/mL]	Area	Amt/Area	Ref	Grp	Name
3.006	1	1	0.00000	1.00000	0.00000			Steviolbioside
3.852	1	1	0.00000	1.00000	0.00000			Rebaudioside B
4.143	1	1	0.00000	1.00000	0.00000			Dulcoside A
4.466	1	1	0.00000	1.00000	0.00000			Stevioside
4.918	1	1	0.00000	1.00000	0.00000			Rebaudioside C
5.058	1	3	4.17872e-1	478.74072	8.72856e-4			Rebaudioside A
		2	1.04468	1192.86121	8.75776e-4			
		1	2.08936	2362.27942	8.84467e-4			
5.800	1	1	0.00000	1.00000	0.00000			Unknown

000102

Sample Name: Rebaudioside Stk

Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min
Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Ret. Times	:	No, only for identified peaks
Curve Type	:	Average Response/Amount
Origin	:	Ignored
Weight	:	Equal

1 Warnings or Errors :

Warning : Overlapping peak time windows at 4.918 min, signal 1

==========

External Standard Report (Sample Amount is 0!) (after recalibration)

==========

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:03:29 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.059	MF	2362.25586	8.77672e-4	2.07329		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07329		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

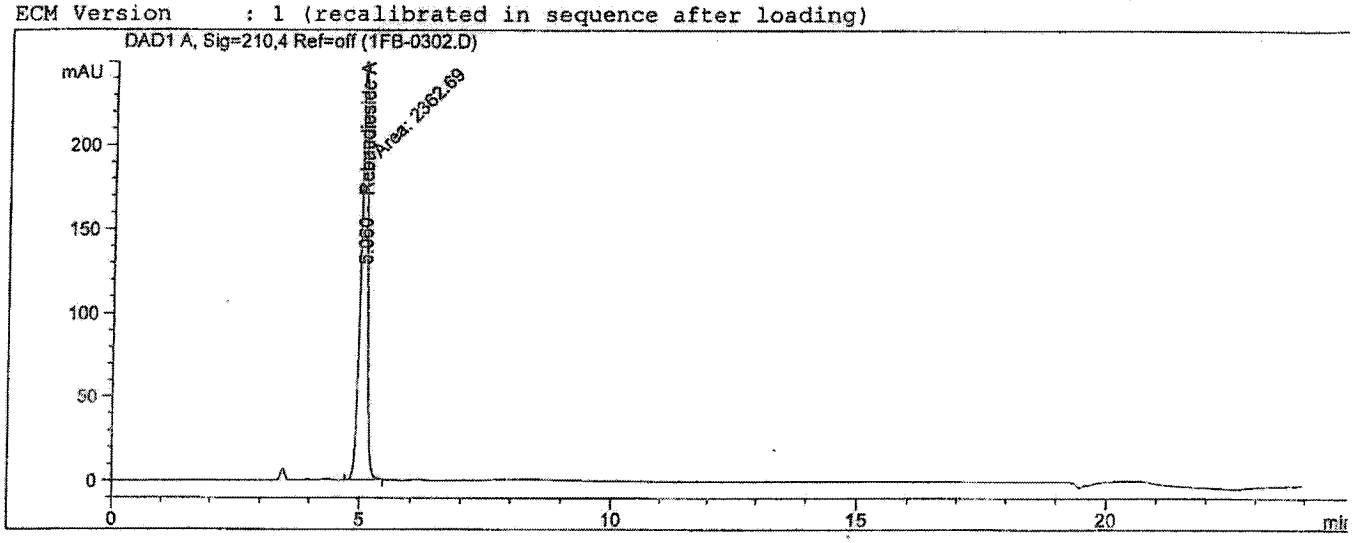
*** End of Report ***

000103

Sample Name: Rebaudioside Stk

```
================================================================================
Acq. Operator   : Mariel Esguerra                  Seq. Line :   3
Acq. Instrument : HPLC 10                           Location : P1-F-02
Injection Date  : 10/18/2010 9:18:09 PM                 Inj :   2
                                                  Inj Volume : 5.0 µl
Sequence File   : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA-7941.S
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 10/20/2010 12:03:34 PM by Mariel Esguerra
                  (recalibrated in sequence after loading)
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sq1c/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version     : 1 (recalibrated in sequence after loading)
```




```
================================================================================
                Calibration Table (after recalibration)
================================================================================

Calib. Data Modified  :      Wednesday, October 20, 2010 12:03:34 PM

Level 1  calibrated: Average Response Factors of all calibrations,
                     Floating Average of Retention Times, New 75%

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Sig	Lvl	Amount [mg/mL]	Area	Amt/Area	Ref	Grp	Name
3.006	1	1	0.00000	1.00000	0.00000			Steviolbioside
3.852	1	1	0.00000	1.00000	0.00000			Rebaudioside B
4.143	1	1	0.00000	1.00000	0.00000			Dulcoside A
4.466	1	1	0.00000	1.00000	0.00000			Stevioside
4.918	1	1	0.00000	1.00000	0.00000			Rebaudioside C
5.060	1	3	4.17872e-1	478.74072	8.72856e-4			Rebaudioside A
		2	1.04468	1192.86121	8.75776e-4			
		1	2.08936	2362.41781	8.84415e-4			
5.800	1	1	0.00000	1.00000	0.00000			Unknown

000104

Sample Name: Rebaudioside Stk

Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min
Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Ret. Times:		No, only for identified peaks

Curve Type	:	Average Response/Amount
Origin	:	Ignored
Weight	:	Equal

1 Warnings or Errors :

Warning : Overlapping peak time windows at 4.918 min, signal 1

==========

External Standard Report (Sample Amount is 0!) (after recalibration)

==========

Sorted By	:	Signal
Calib. Data Modified	:	Wednesday, October 20, 2010 12:03:34 PM
Multiplier:	:	1.0000
Dilution:	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.060	MM	2362.69458	8.77655e-4	2.07363		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07363		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

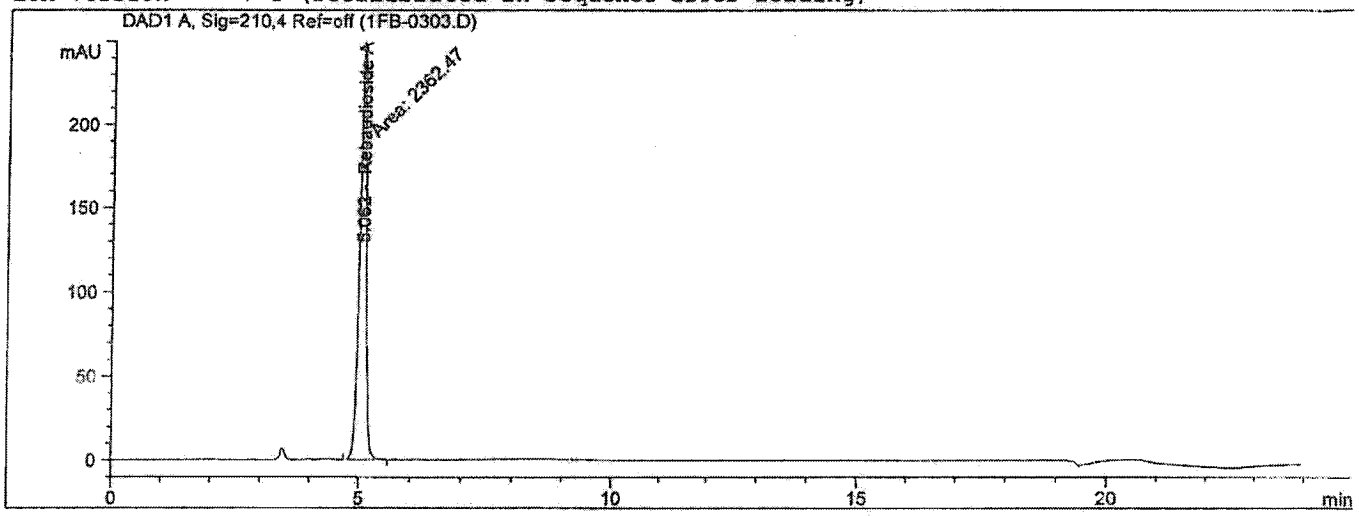
==========

*** End of Report ***

Data File C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\1FB-0303.D
Sample Name: Rebaudioside Stk

Acq. Operator	: Mariel Esguerra	Seq. Line	: 3
Acq. Instrument	: HPLC 10	Location	: P1-F-02
Injection Date	: 10/18/2010 9:57:52 PM	Inj	: 3
		Inj Volume	: 5.0 µl
Sequence File	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA-7941.S	
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA.M	
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA.M	
Last changed	: 10/20/2010 12:03:40 PM by Mariel Esguerra (recalibrated in sequence after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server	: http://us05sqlc/ecmwg
ECM Operator	: Mariel Esguerra
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version	: 1 (recalibrated in sequence after loading)



Calibration Table (after recalibration)

Calib. Data Modified : Wednesday, October 20, 2010 12:03:40 PM

Level 1 calibrated: Average Response Factors of all calibrations,
Floating Average of Retention Times, New 75%

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Sig	Lvl	Amount [mg/mL]	Area	Amt/Area	Ref Grp	Name
3.006	1	1	0.00000	1.00000	0.00000		Steviolbioside
3.852	1	1	0.00000	1.00000	0.00000		Rebaudioside B
4.143	1	1	0.00000	1.00000	0.00000		Dulcoside A
4.466	1	1	0.00000	1.00000	0.00000		Stevioside
4.918	1	1	0.00000	1.00000	0.00000		Rebaudioside C
5.061	1	3	4.17872e-1	478.74072	8.72856e-4		Rebaudioside A
		2	1.04468	1192.86121	8.75776e-4		
		1	2.08936	2362.43024	8.84410e-4		
5.800	1	1	0.00000	1.00000	0.00000		Unknown

000106

Data File C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\REB-0303.D
Sample Name: Rebaudioside Stk

Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min
Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Ret. Times	:	No, only for identified peaks
Curve Type	:	Average Response/Amount
Origin	:	Ignored
Weight	:	Equal

1 Warnings or Errors :

Warning : Overlapping peak time windows at 4.918 min, signal 1

==========

External Standard Report (Sample Amount is 0!) (after recalibration)

==========

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:03:40 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.062	MM	2362.46753	8.77653e-4	2.07343		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07343		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

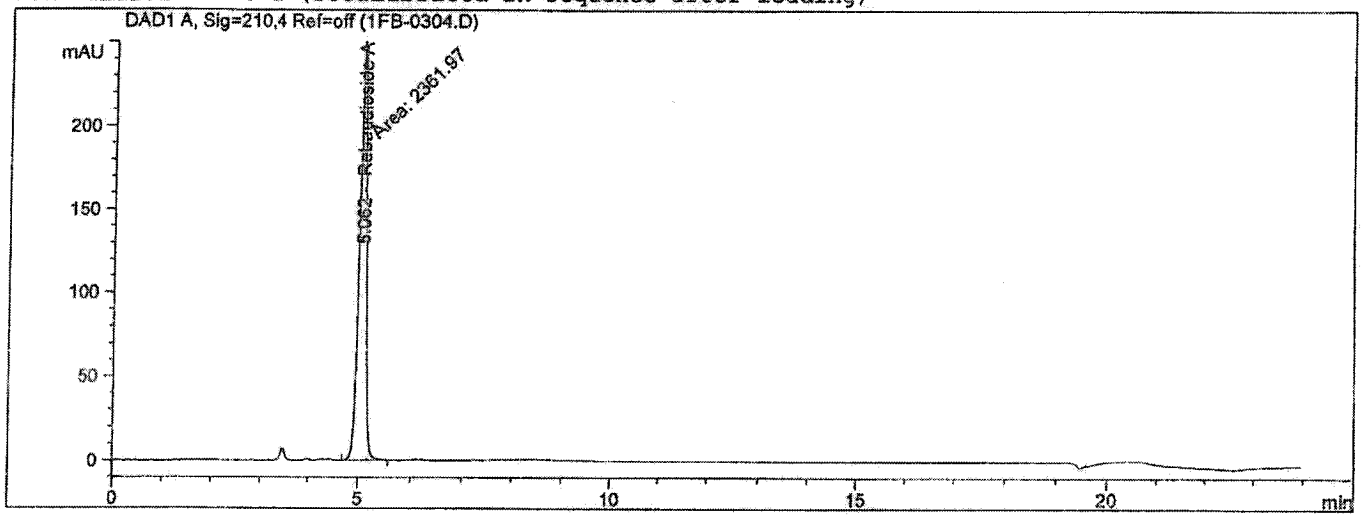
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

Acq. Operator	: Mariel Esguerra	Seq. Line	: 3
Acq. Instrument	: HPLC 10	Location	: P1-F-02
Injection Date	: 10/18/2010 10:37:36 PM	Inj	: 4
		Inj Volume	: 5.0 µl
Sequence File	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA-7941.S	
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA.M	
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941	2010-10-18\STEVIA.M	
Last changed	: 10/20/2010 12:03:45 PM by Mariel Esguerra (recalibrated in sequence after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (recalibrated in sequence after loading)



Calibration Table (after recalibration)

Calib. Data Modified : Wednesday, October 20, 2010 12:03:45 PM

Level 1 calibrated: Average Response Factors of all calibrations,
Floating Average of Retention Times, New 75%

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Sig	Lvl	Amount [mg/mL]	Area	Amt/Area	Ref	Grp	Name
3.006	1	1	0.00000	1.00000	0.00000			Steviolbioside
3.852	1	1	0.00000	1.00000	0.00000			Rebaudioside B
4.143	1	1	0.00000	1.00000	0.00000			Dulcoside A
4.466	1	1	0.00000	1.00000	0.00000			Stevioside
4.918	1	1	0.00000	1.00000	0.00000			Rebaudioside C
5.062	1	3	4.17872e-1	478.74072	8.72856e-4			Rebaudioside A
		2	1.04468	1192.86121	8.75776e-4			
		1	2.08936	2362.33823	8.84445e-4			
5.800	1	1	0.00000	1.00000	0.00000			Unknown

000108

Sample Name: Rebaudioside Stk

Rel. Reference Window	:	5.000 %
Abs. Reference Window	:	0.000 min
Rel. Non-ref. Window	:	5.000 %
Abs. Non-ref. Window	:	0.000 min
Uncalibrated Peaks	:	not reported
Partial Calibration	:	Yes, identified peaks are recalibrated
Correct All Ret. Times:		No, only for identified peaks

Curve Type	:	Average Response/Amount
Origin	:	Ignored
Weight	:	Equal

1 Warnings or Errors :

Warning : Overlapping peak time windows at 4.918 min, signal 1

==========

External Standard Report (Sample Amount is 0!) (after recalibration)

==========

Sorted By	:	Signal
Calib. Data Modified	:	Wednesday, October 20, 2010 12:03:45 PM
Multiplier:	:	1.0000
Dilution:	:	1.0000

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.062	MM	2361.97021	8.77665e-4	2.07302		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07302		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

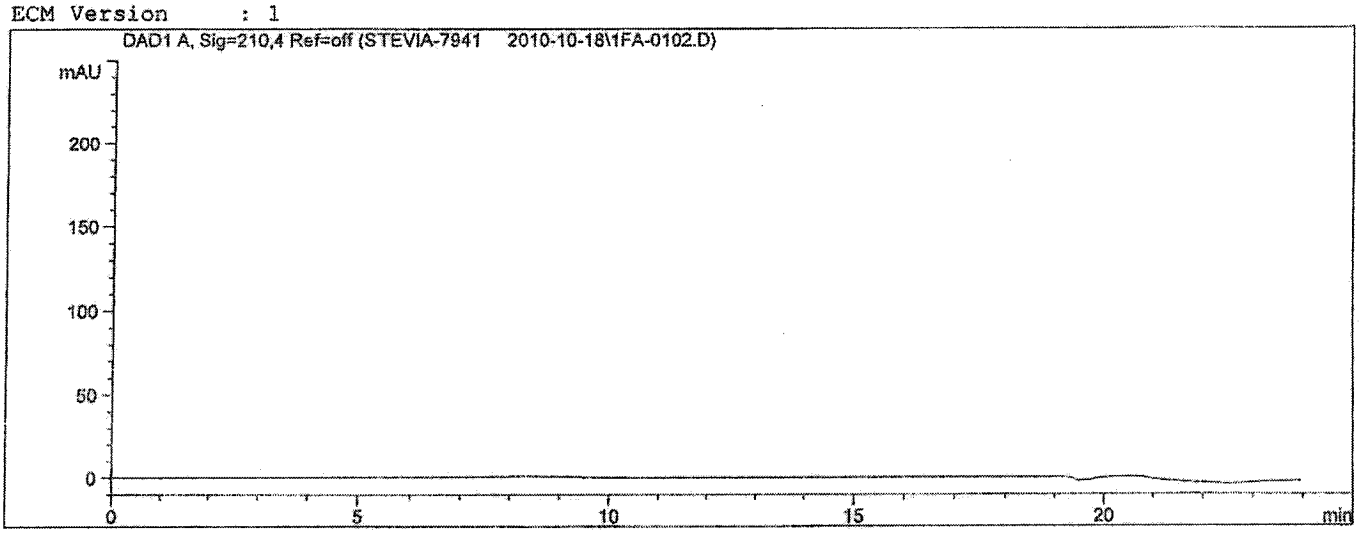
Appendix #3

Selectivity
(Solvent Blank)

Sample Name: Blank

Acq. Operator	: Mariel Esguerra	Seq. Line	: 1
Acq. Instrument	: HPLC 10	Location	: P1-F-01
Injection Date	: 10/18/2010 7:19:00 PM	Inj	: 2
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 11:18:07 AM by Mariel Esguerra		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : 10/20/2010 11:17:58 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.053		-	-	-		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				0.00000		

000111

Data File C:\CHEM32\1\DATA\STEVIA-7941 2010-10-18\1FA-0102.D
Sample Name: Blank

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 10/20/2010 11:17:58 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.006		0.0000	0.00000	0.0000	Steviolbioside
2	3.852		0.0000	0.00000	0.0000	Rebaudioside B
3	4.143		0.0000	0.00000	0.0000	Dulcoside A
4	4.466		0.0000	0.00000	0.0000	Stevioside
5	4.918		0.0000	0.00000	0.0000	Rebaudioside C
6	5.053		0.0000	0.00000	0.0000	Rebaudioside A
7	5.800		0.0000	0.00000	0.0000	Unknown
Totals :				0.00000		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

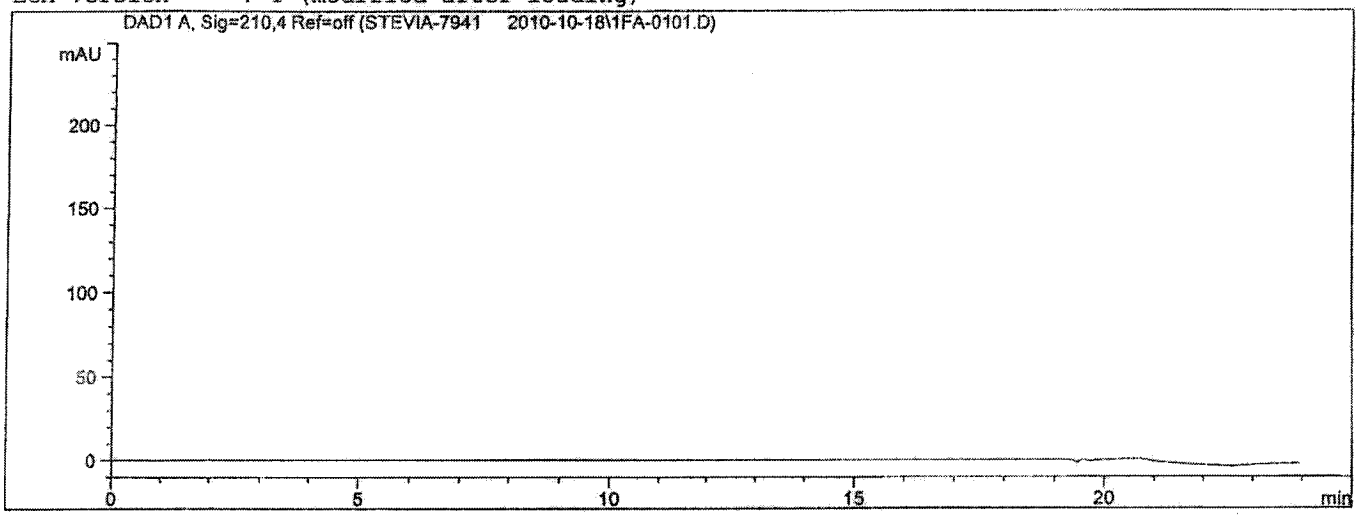
==========

*** End of Report ***

000112

Data File C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\1FA-0101.D
Sample Name: Blank

Acq. Operator	: Mariel Esguerra	Seq. Line	: 1
Acq. Instrument	: HPLC 10	Location	: P1-F-01
Injection Date	: 10/18/2010 6:39:17 PM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 11:17:56 AM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : 10/20/2010 11:17:58 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.053		-	-	-		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				0.00000		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

Area Percent Report

==========

Sorted By : Signal
Calib. Data Modified : 10/20/2010 11:17:58 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
1	3.006		0.0000	0.00000	0.0000	Steviolbioside
2	3.852		0.0000	0.00000	0.0000	Rebaudioside B
3	4.143		0.0000	0.00000	0.0000	Dulcoside A
4	4.466		0.0000	0.00000	0.0000	Stevioside
5	4.918		0.0000	0.00000	0.0000	Rebaudioside C
6	5.053		0.0000	0.00000	0.0000	Rebaudioside A
7	5.800		0.0000	0.00000	0.0000	Unknown
Totals :				0.00000		

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

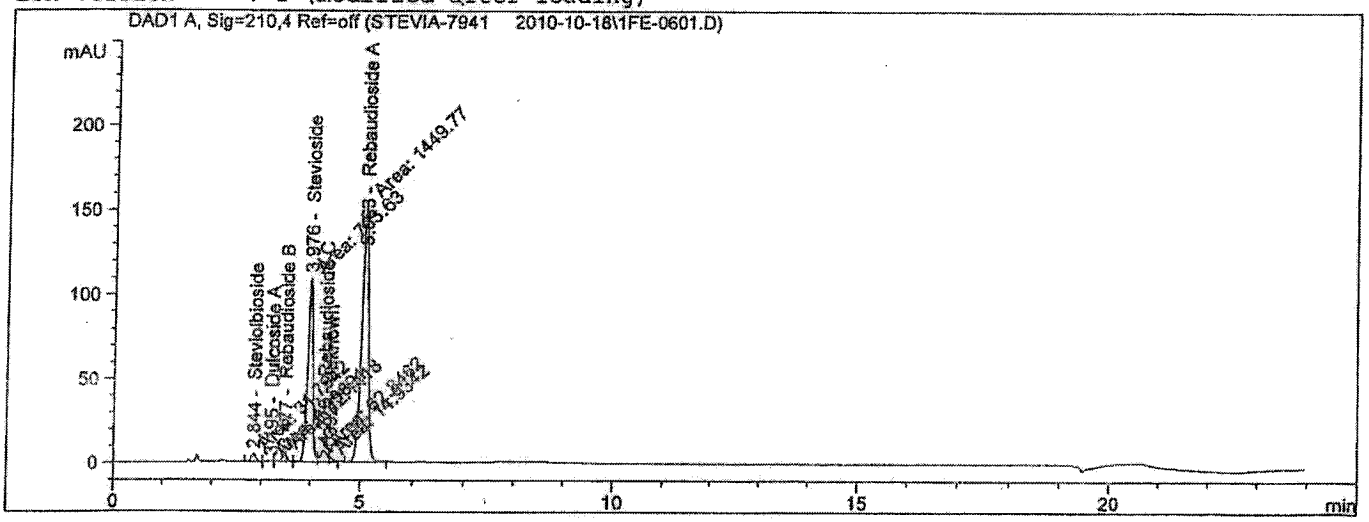
Appendix #4

Accuracy/Selectivity (Positive Control)

Sample Name: Control new

Acq. Operator	: Mariel Esguerra	Seq. Line	: 6
Acq. Instrument	: HPLC 10	Location	: P1-F-05
Injection Date	: 10/19/2010 12:36:40 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:37:41 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (modified after loading)



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:34:47 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.00350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.844	MF	31.79421	5.83724e-4	0.926331		Steviolbioside
3.195	MF	4.09891	7.25233e-4	0.148374		Dulcoside A
3.477	MF	42.14180	7.34077e-4	1.544064		Rebaudioside B
3.976	MF	793.63043	7.34077e-4	29.078405		Stevioside
4.279	MF	62.84620	8.66741e-4	2.718811		Rebaudioside C
4.392	MF	14.93418	8.84430e-4	0.659258		Unknown
5.063	MF	1449.76501	8.84433e-4	63.999018		Rebaudioside A

Sample Name: Control new

Totals : 99.074261

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

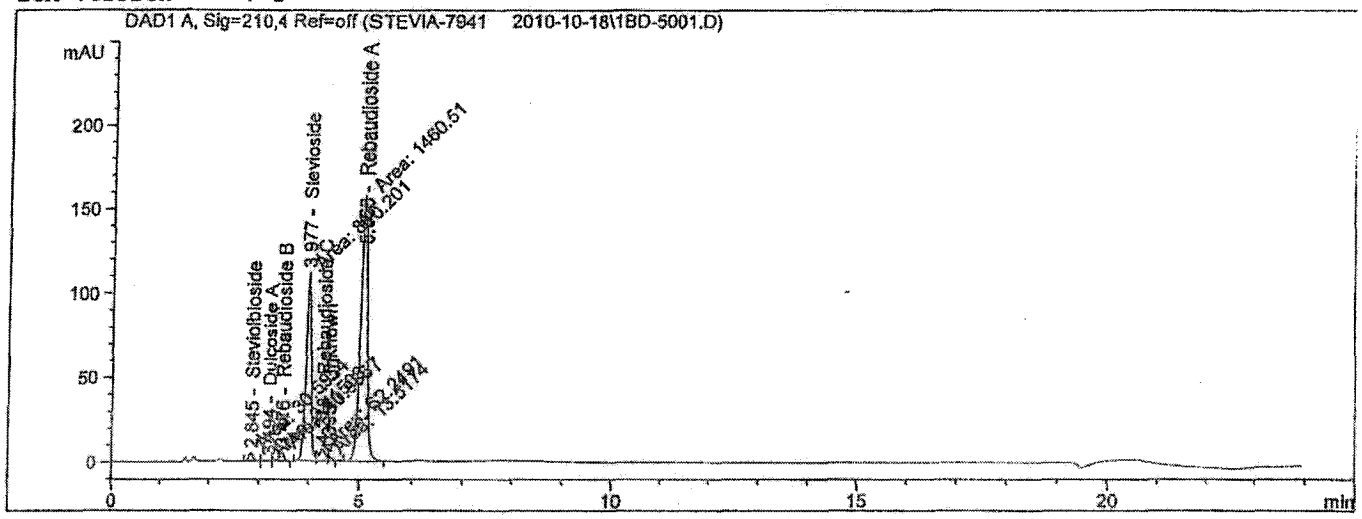
==========

*** End of Report ***

Sample Name: Control old

Acq. Operator	: (b) (6) i	Seq. Line	: 50
Acq. Instrument	: HPLC 10	Location	: P1-B-04
Injection Date	: 10/20/2010 5:44:43 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:37:56 PM by Mariel Esguerra		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:34:47 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.00350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.845	MF	30.59544	5.83724e-4	0.891405		Steviolbioside
3.194	MF	3.41503	7.25233e-4	0.123618		Dulcoside A
3.476	MF	40.93667	7.34077e-4	1.499909		Rebaudioside B
3.977	FM	800.20105	7.34077e-4	29.319151		Stevioside
4.278	MF	62.24906	8.66741e-4	2.692978		Rebaudioside C
4.395	MF	13.51745	8.84430e-4	0.596718		Unknown
5.063	MF	1460.51331	8.84433e-4	64.473495		Rebaudioside A
Totals :				99.597273		

Sample Name: Control old

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

==========

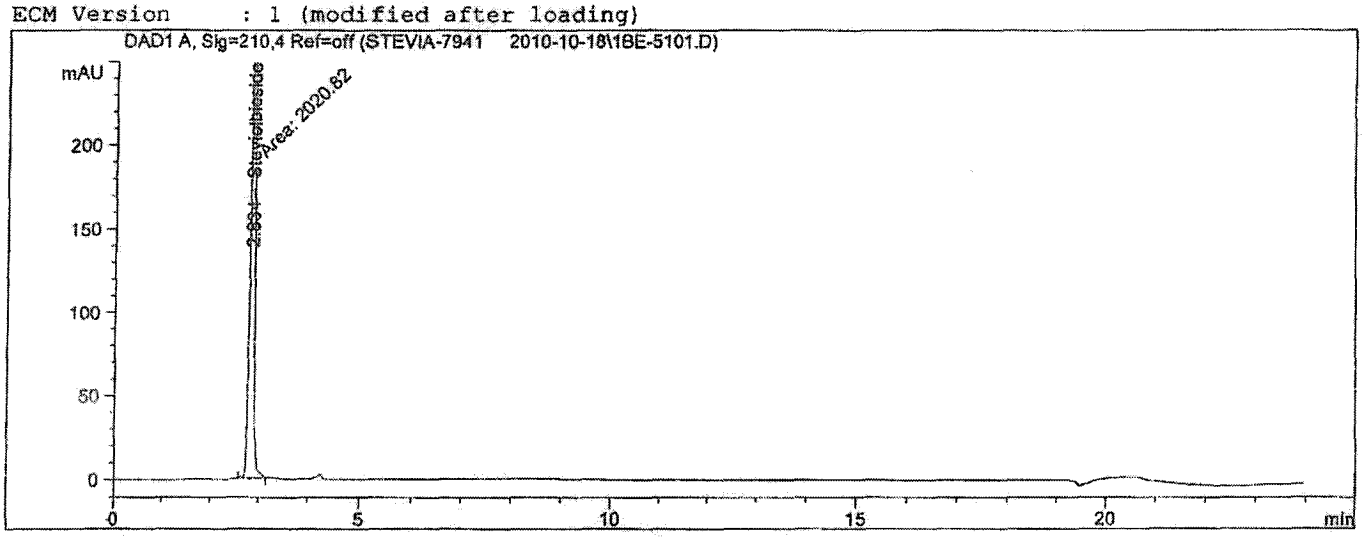
*** End of Report ***

Appendix #5

Retention Time Markers

Data File C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\1BE-5101.D
Sample Name: Steviolbioside

Acq. Operator	: (b) (6)	Seq. Line	: 51
Acq. Instrument	: HPLC 10	Location	: P1-B-05
Injection Date	: 10/20/2010 6:24:28 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:12:09 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal	
Calib. Data Modified	:	10/20/2010 12:12:09 PM	
Multiplier:	:	1.0000	
Dilution:	:	1.0000	

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
2.834	MM	2020.81689	0.00000	0.00000		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.060		-	-	-		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				0.00000		

Data File C:\CHEM32\1\DATA\STEVIA-7941 2010-10-18\1BE-5101.D
Sample Name: Steviolbioside

3 Warnings or Errors :

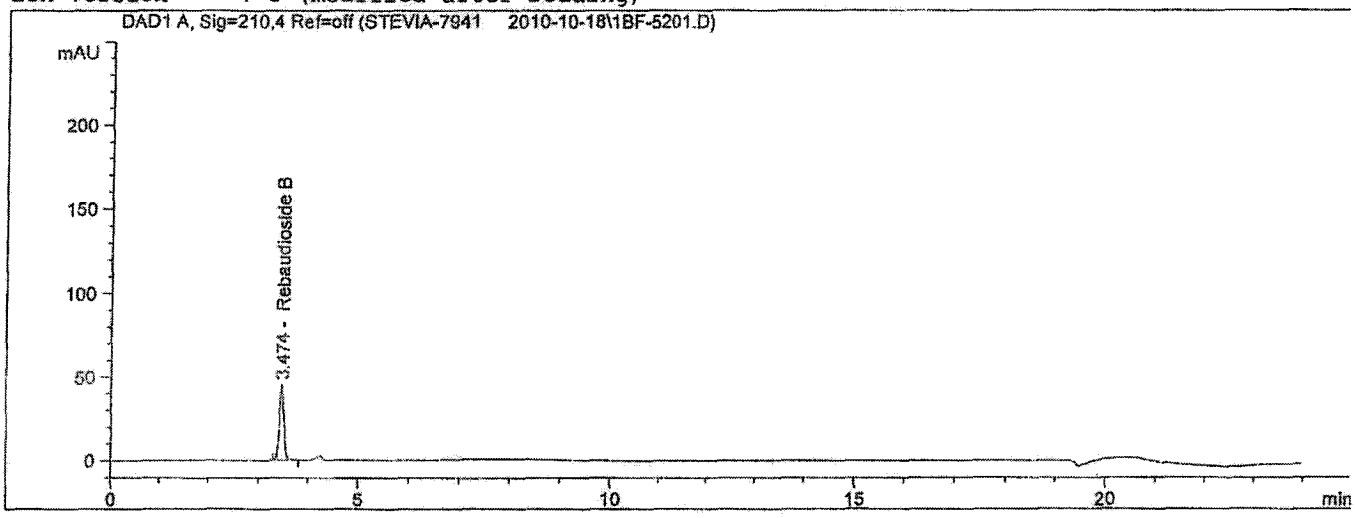
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Steviolbioside)

==========

*** End of Report ***

Sample Name: Rebaudioside B

Acq. Operator	: (b) (6) i	Seq. Line	: 52
Acq. Instrument	: HPLC 10	Location	: P1-B-06
Injection Date	: 10/20/2010 7:04:12 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:12:48 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:12:48 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
2.834		-	-	-		Steviolbioside
3.474	BB	285.17950	0.00000	0.00000		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.060		-	-	-		Rebaudioside A
5.800		-	-	-		Unknown
Totals :			0.00000			

Sample Name: Rebaudioside B

3 Warnings or Errors :

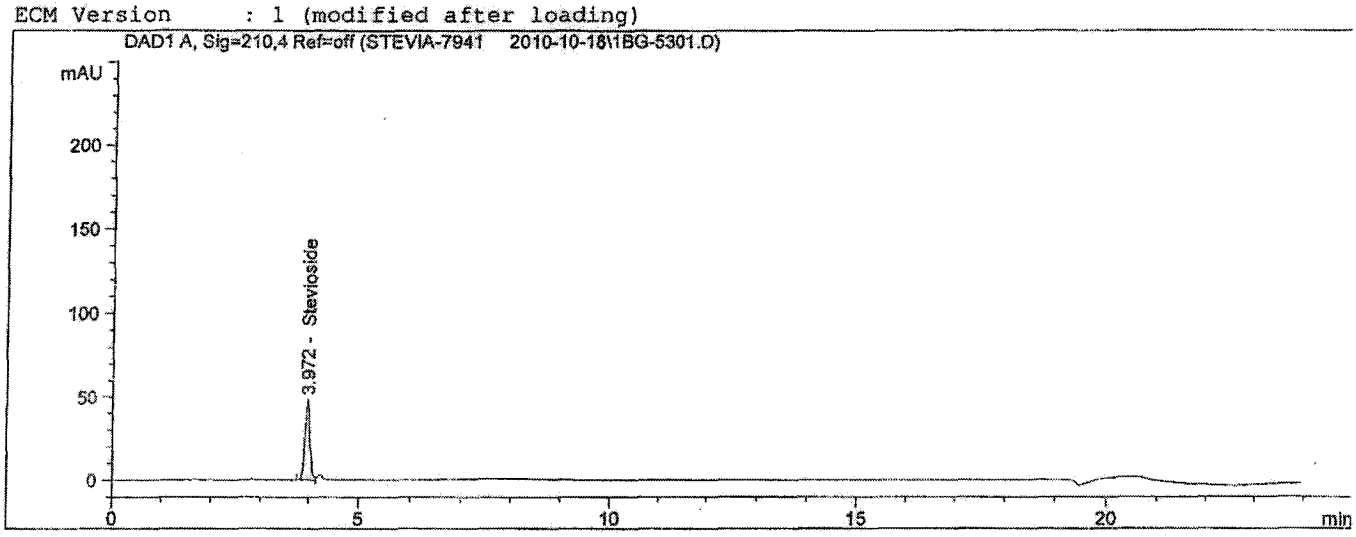
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Rebaudioside B)

==========

*** End of Report ***

Sample Name: Stevioside

Acq. Operator	: (b) (6)	Seq. Line	: 53
Acq. Instrument	: [illegible]	Location	: P1-B-07
Injection Date	: 10/20/2010 7:43:57 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:13:28 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:13:28 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
2.834		-	-	-		Steviolbioside
3.474		-	-	-		Rebaudioside B
3.972	BV	347.62439	0.00000	0.00000		Stevioside
4.143		-	-	-		Dulcoside A
4.918		-	-	-		Rebaudioside C
5.060		-	-	-		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				0.00000		

000125

Sample Name: Stevioside

3 Warnings or Errors :

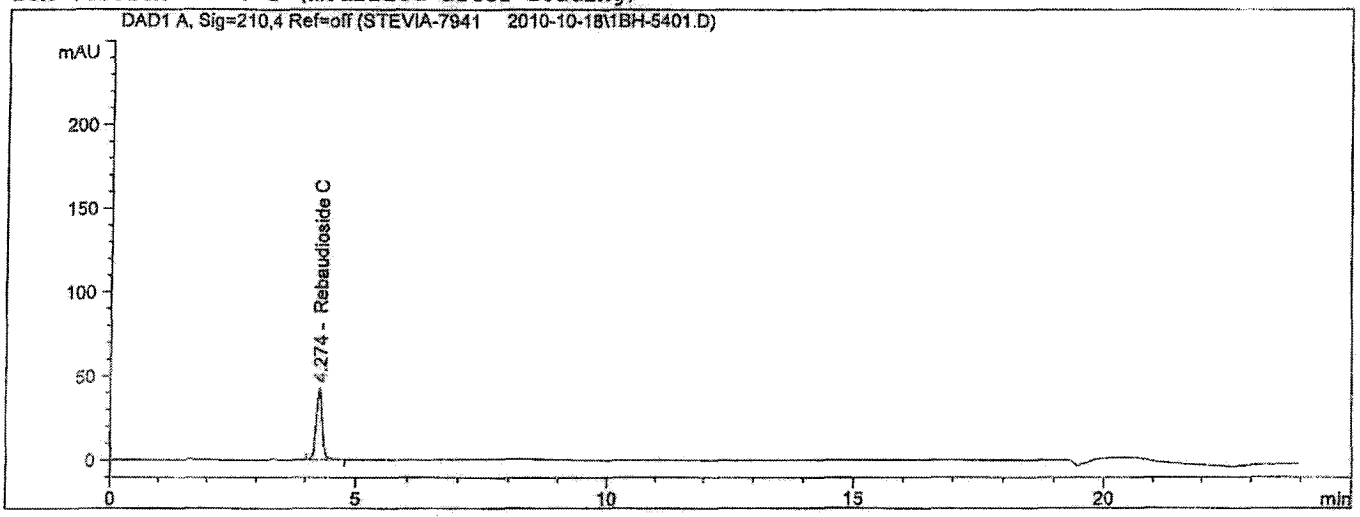
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Stevioside)

==========

*** End of Report ***

Sample Name: Rebaudioside C

Acq. Operator	: (b) (6)	Seq. Line	: 54
Acq. Instrument	: HPLC 10	Location	: P1-B-08
Injection Date	: 10/20/2010 8:23:41 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:14:17 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:14:16 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
2.834		-	-	-		Steviolbioside
3.474		-	-	-		Rebaudioside B
3.972		-	-	-		Stevioside
4.143		-	-	-		Dulcoside A
4.274	BB	354.32574	0.00000	0.00000		Rebaudioside C
5.060		-	-	-		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				0.00000		

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Rebaudioside C)

==========

*** End of Report ***

Appendix #6

Reference Standard System Suitability (RSD)

Statistic Report

Bracketing Standards only
mze 08Dec10

Sequence table: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA-7941.S
Data directory path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Ver: 1
Operator: Mariel Esguerra

Method file name: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M

Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	P1-F-08	1	10/19/2010 2:35:50 AM	1FH-0901.D	Rebaudioside Stk
2	P1-F-08	1	10/19/2010 5:14:42 AM	1FH-1301.D	Rebaudioside Stk
3	P1-F-08	1	10/19/2010 7:53:36 AM	1FH-1701.D	Rebaudioside Stk
4	P1-F-08	1	10/19/2010 10:32:32 AM	1FH-2101.D	Rebaudioside Stk
5	P1-F-08	1	10/19/2010 1:11:27 PM	1FH-2501.D	Rebaudioside Stk
6	P1-D-06	1	10/19/2010 3:50:21 PM	1DF-2901.D	Rebaudioside Stk
7	P1-D-06	1	10/19/2010 6:29:16 PM	1DF-3301.D	Rebaudioside Stk
8	P1-D-06	1	10/19/2010 9:08:10 PM	1DF-3701.D	Rebaudioside Stk
9	P1-D-06	1	10/19/2010 11:47:07 PM	1DF-4101.D	Rebaudioside Stk
10	P1-D-06	1	10/20/2010 2:26:04 AM	1DF-4501.D	Rebaudioside Stk
11	P1-D-06	1	10/20/2010 5:04:59 AM	1DF-4901.D	Rebaudioside Stk
12	P1-D-06	1	10/20/2010 9:03:26 AM	1DF-5501.D	Rebaudioside Stk

Statistic results for compound Steviolbioside not available.

Statistic results for compound Dulcoside A not available.

Statistic results for compound Rebaudioside B not available.

Statistic results for compound Stevioside not available.

Statistic results for compound Rebaudioside C not available.

Statistic results for compound Unknown not available.

Compound: Rebaudioside A (Signal: DAD1 A, Sig=210,4 Ref=off)

Run #	Type	RetTime [min]	Amount [mg/mL]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	MM	5.066	2.08976	2362.84985	253.48116	0.1400	1.07
2	MF	5.065	2.08950	2362.56714	254.23129	0.1383	1.06
3	MM	5.064	2.08884	2361.79150	254.65474	0.1383	1.06
4	MM	5.068	2.08950	2362.53833	254.62416	0.1383	1.06
5	MM	5.077	2.08995	2363.08594	254.22134	0.1383	1.06
6	MM	5.070	2.08889	2361.86304	254.88712	0.1400	1.06
7	MM	5.067	2.08974	2362.84155	254.63269	0.1400	1.06
8	MM	5.067	2.08887	2361.83228	254.88556	0.1383	1.06
9	BB	5.064	2.08969	2362.77563	255.22141	0.1383	1.06
10	MM	5.063	2.08878	2361.71802	255.60187	0.1383	1.06
11	MM	5.060	2.08944	2362.47607	255.92769	0.1400	1.06
12	MM	5.058	2.08924	2362.24634	256.51361	0.1400	1.06

	RetTime [min]	Amount [mg/mL]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
Mean:	5.066	2.08935	2362.38214	254.90689	0.1390	1.06
S.D.:	4.80e-3	4.14578e-4	4.79356e-1	8.19322e-1	8.58e-4	2e-3
RSD :	0.095	1.98424e-2	2.02912e-2	3.21420e-1	0.6173	0.21
95% CI:	3.05e-3	2.63410e-4	3.04568e-1	5.20572e-1	5.45e-4	1e-3

Statistic Report

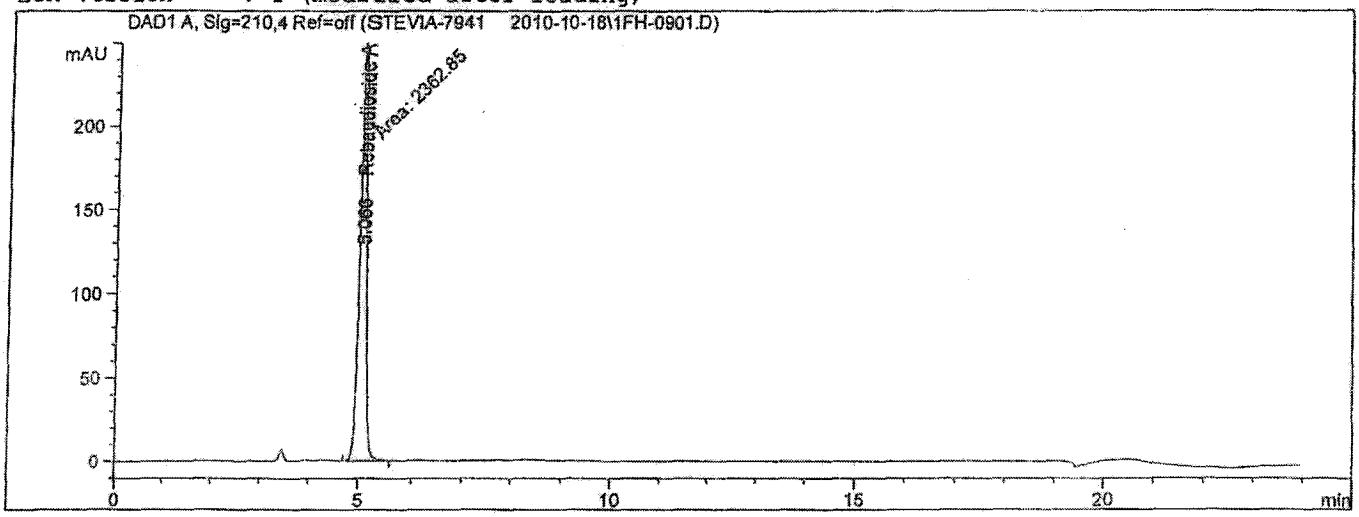
Sequence table: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA-7941.S
Data directory path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Ver: 1
Operator: Mariel Esguerra

No (or not enough) sample runs with cal. compounds available!

Sample Name: Rebaudioside Stk

Acq. Operator	: Mariel Esguerra	Seq. Line	: 9
Acq. Instrument	: HPLC 10	Location	: P1-F-08
Injection Date	: 10/19/2010 2:35:50 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:05:37 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (modified after loading)



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:05:36 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.066	MM	2362.84985	8.77654e-4	2.07376		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07376		

000133

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

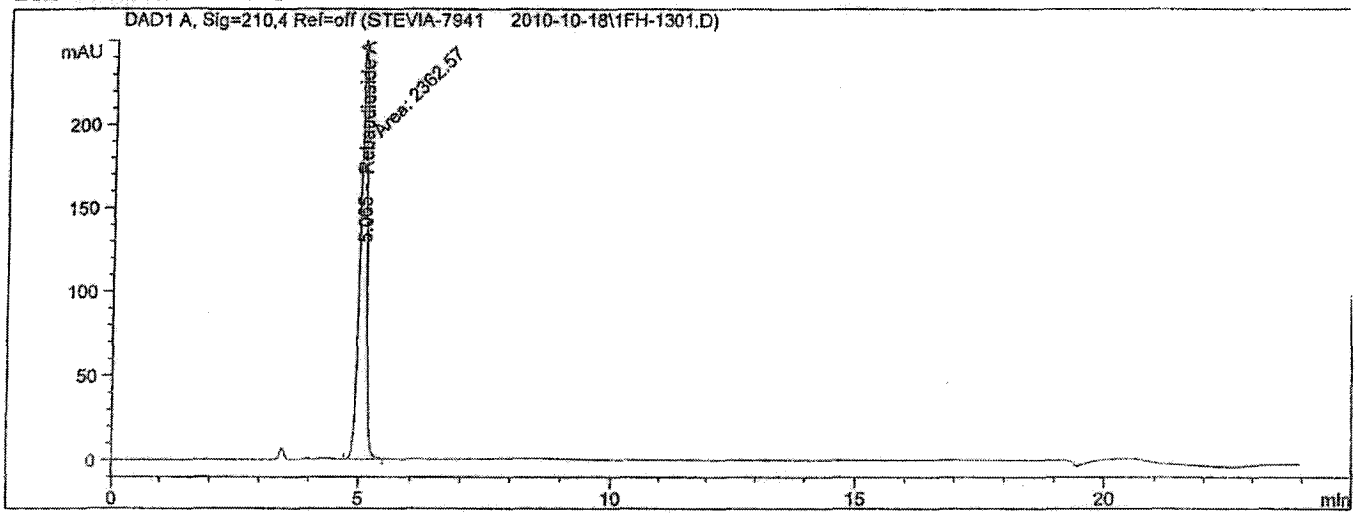
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

```
Acq. Operator    : Mariel Esguerra                Seq. Line :  13
Acq. Instrument  : HPLC 10                         Location : P1-F-08
Injection Date   : 10/19/2010 5:14:42 AM                Inj :   1
                                                 Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 12:05:44 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:05:36 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.065	MF	2362.56714	8.77654e-4	2.07352		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07352		

000135

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

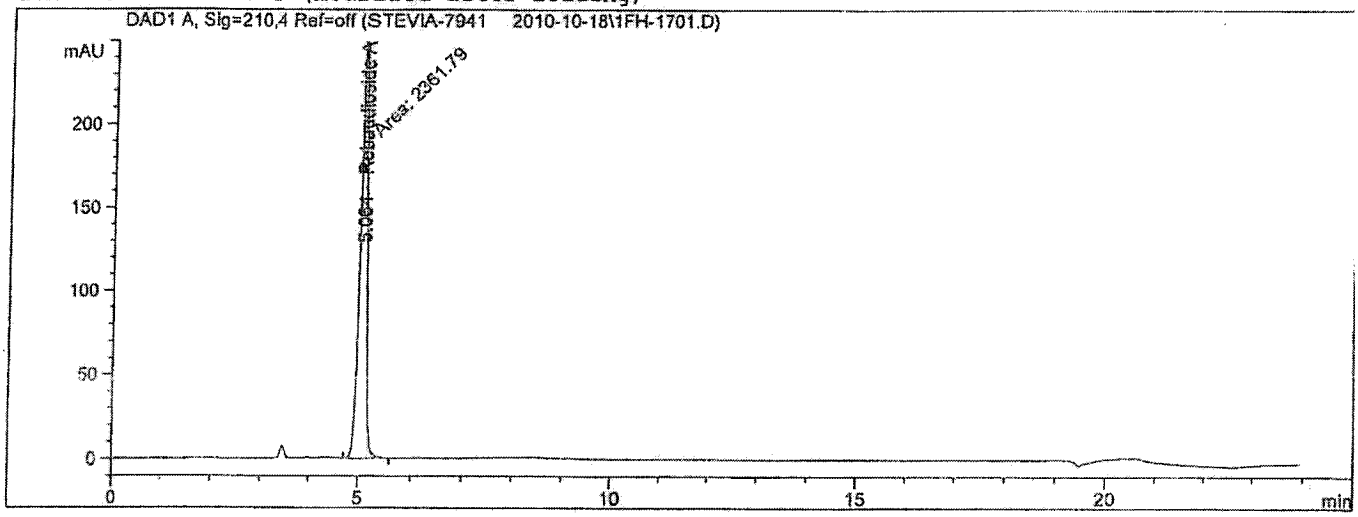
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

```
Acq. Operator    : Mariel Esguerra                Seq. Line :  17
Acq. Instrument  : HPLC 10                         Location : P1-F-08
Injection Date   : 10/19/2010 7:53:36 AM                Inj :  1
                                                 Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 12:07:46 PM by Mariel Esguerra
                   (modified after loading)
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1 (modified after loading)
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:07:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.064	MM	2361.79150	8.77661e-4	2.07285		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07285		

000137

2 Warnings or Errors :

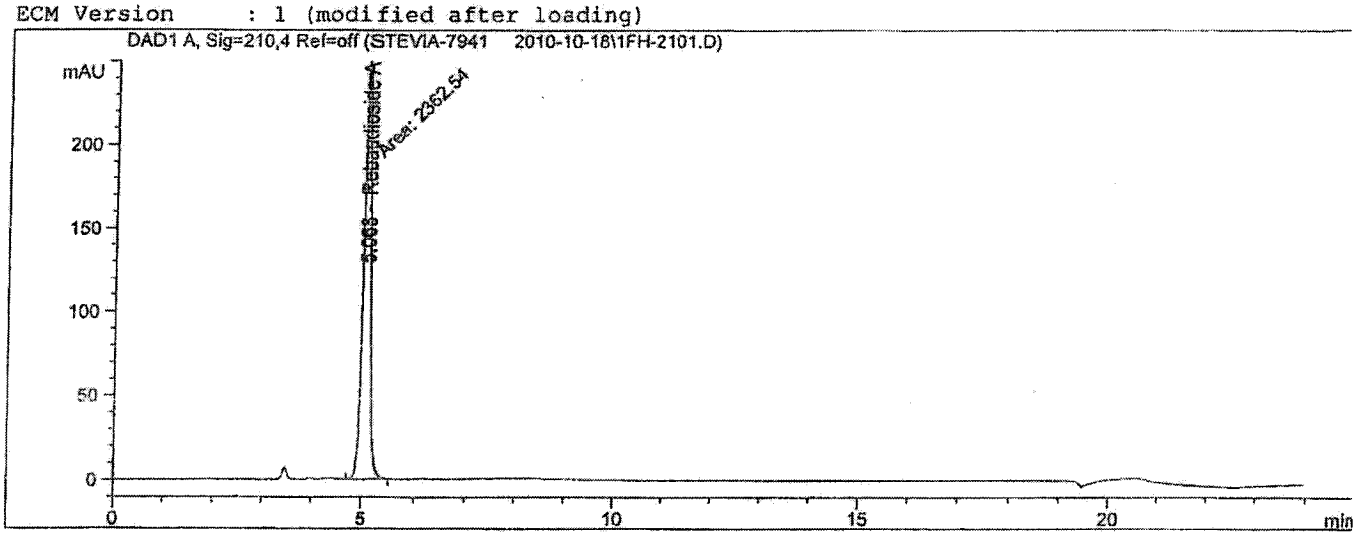
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Sample Name: Rebaudioside Stk

Acq. Operator	: (b) (6)	Seq. Line	: 21
Acq. Instrument	: [redacted]	Location	: P1-F-08
Injection Date	: 10/19/2010 10:32:32 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:07:46 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:07:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.068	MM	2362.53833	8.77661e-4	2.07351		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07351		

000139

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

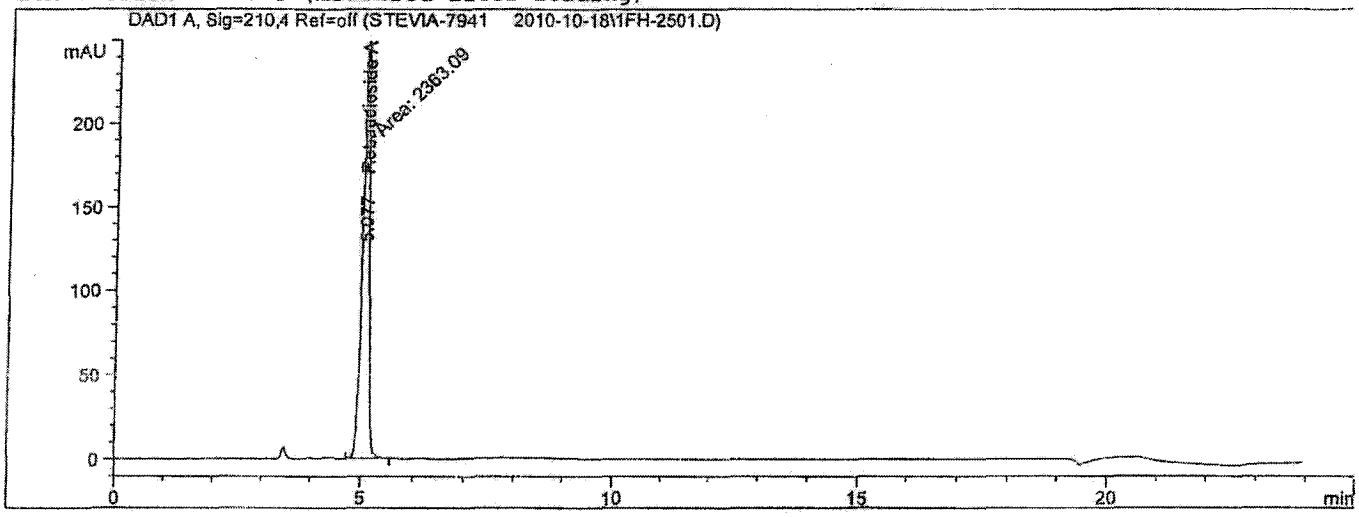
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

Acq. Operator	: (b) (6)	Seq. Line	: 25
Acq. Instrument	: HPLC 10	Location	: P1-F-08
Injection Date	: 10/19/2010 1:11:27 PM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:07:46 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (modified after loading)



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:07:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.077	MM	2363.08594	8.77661e-4	2.07399		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07399		

000141

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

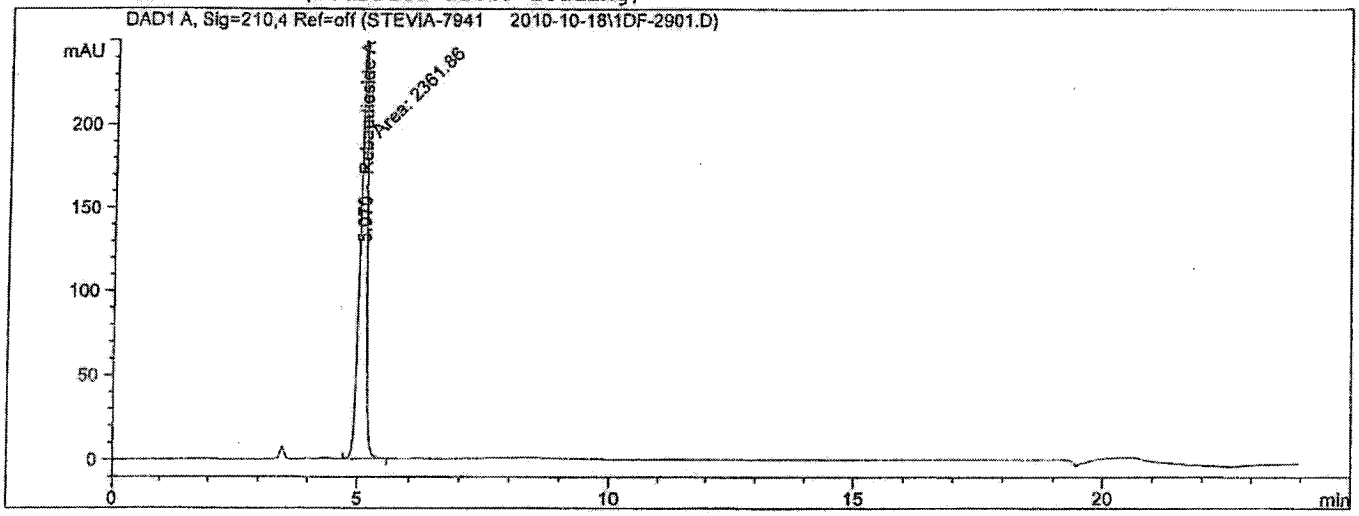
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

```
Acq. Operator   : (b) (6)                    Seq. Line :  29
Acq. Instrument : HPLC 10                    Location : P1-D-06
Injection Date  : 10/19/2010 3:50:21 PM          Inj :   1
                                           Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 10/20/2010 12:07:46 PM by Mariel Esguerra
                  (modified after loading)
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version     : 1 (modified after loading)
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:07:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.070	MM	2361.86304	8.77661e-4	2.07291		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07291		

000143

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

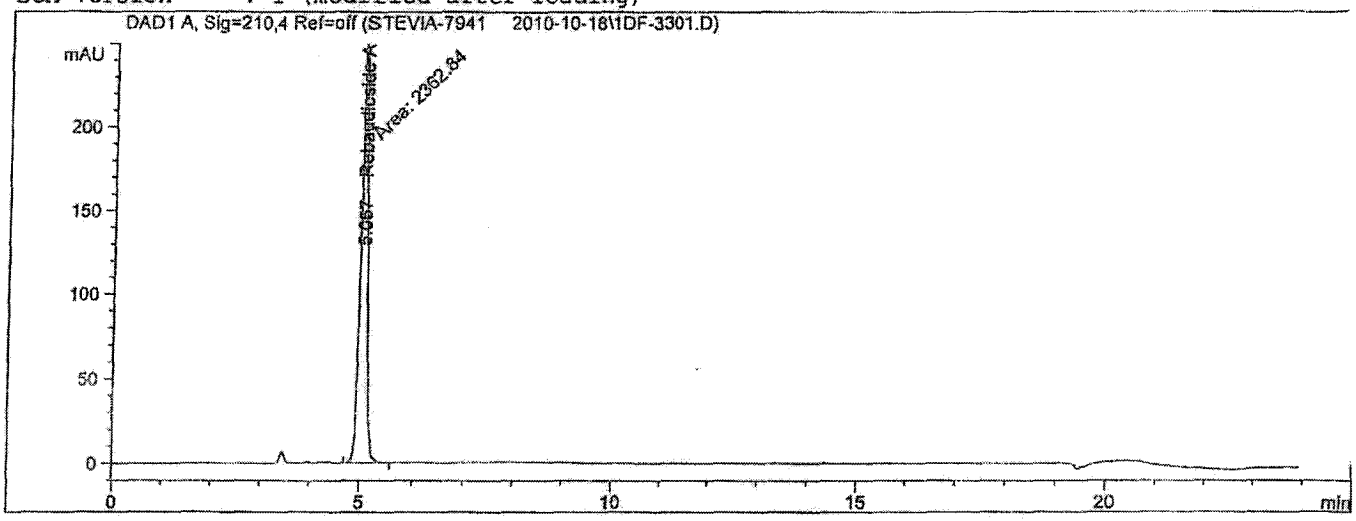
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

```
Acq. Operator    : (b) (6)          i                    Seq. Line :  33
Acq. Instrument  : HPLC 10                               Location : P1-D-06
Injection Date   : 10/19/2010 6:29:16 PM                     Inj :   1
                                                       Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 12:07:46 PM by Mariel Esguerra
                   (modified after loading)
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1 (modified after loading)
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:07:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.067	MM	2362.84155	8.77661e-4	2.07377		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07377		

2 Warnings or Errors :

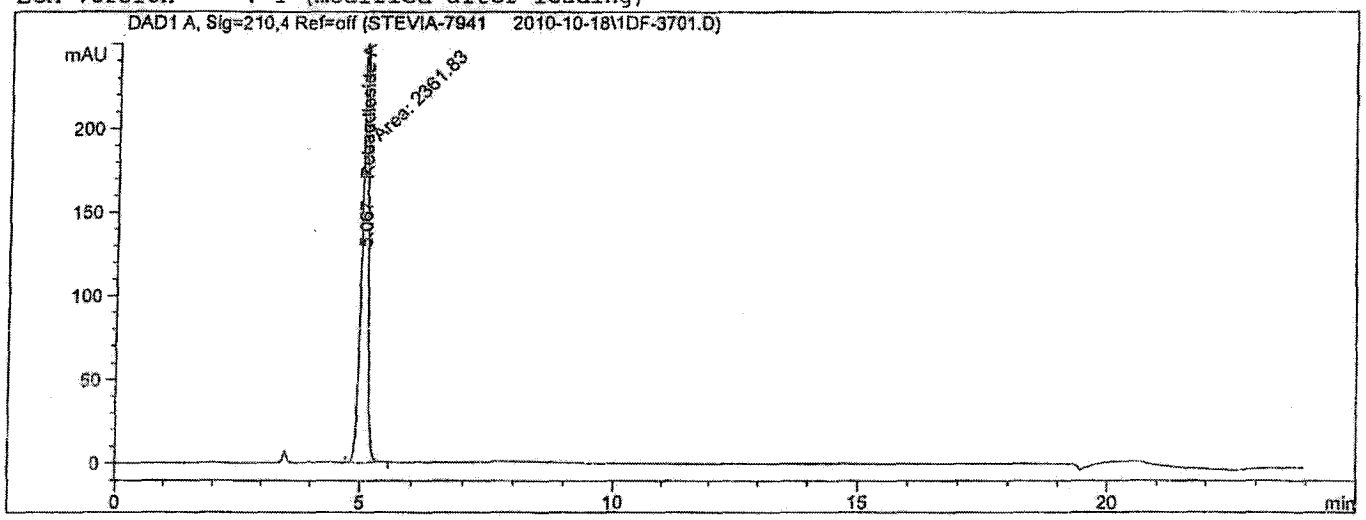
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Sample Name: Rebaudioside Stk

Acq. Operator	: (b) (6)	Seq. Line	: 37
Acq. Instrument	: HPLC 10	Location	: P1-D-06
Injection Date	: 10/19/2010 9:08:10 PM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:07:46 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		
ECM Server	: http://us05sqlc/ecmwg		
ECM Operator	: Mariel Esguerra		
ECM Path	: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip		
ECM Version	: 1 (modified after loading)		



External Standard Report (Sample Amount is 0!)

Sorted By	:	Signal	
Calib. Data Modified	:	Wednesday, October 20, 2010 12:07:45 PM	
Multiplier:	:	1.0000	
Dilution:	:	1.0000	

Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.067	MM	2361.83228	8.77661e-4	2.07289		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07289		

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

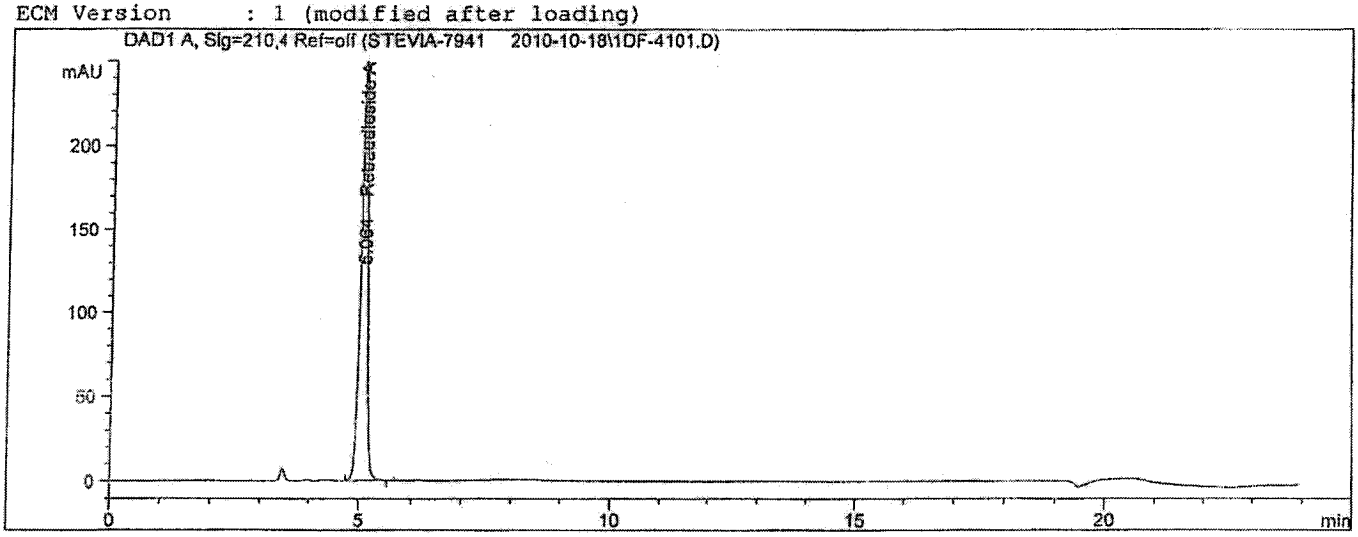
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

Acq. Operator	: (b) (6)	Seq. Line	: 41
Acq. Instrument	: HPLC 10	Location	: P1-D-06
Injection Date	: 10/19/2010 11:47:07 PM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 12:07:46 PM by Mariel Esguerra (modified after loading)		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (modified after loading)



External Standard Report (Sample Amount is 0!)

Sorted By : Signal
Calib. Data Modified : Wednesday, October 20, 2010 12:07:45 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.064	BB	2362.77563	8.77661e-4	2.07372		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07372		

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

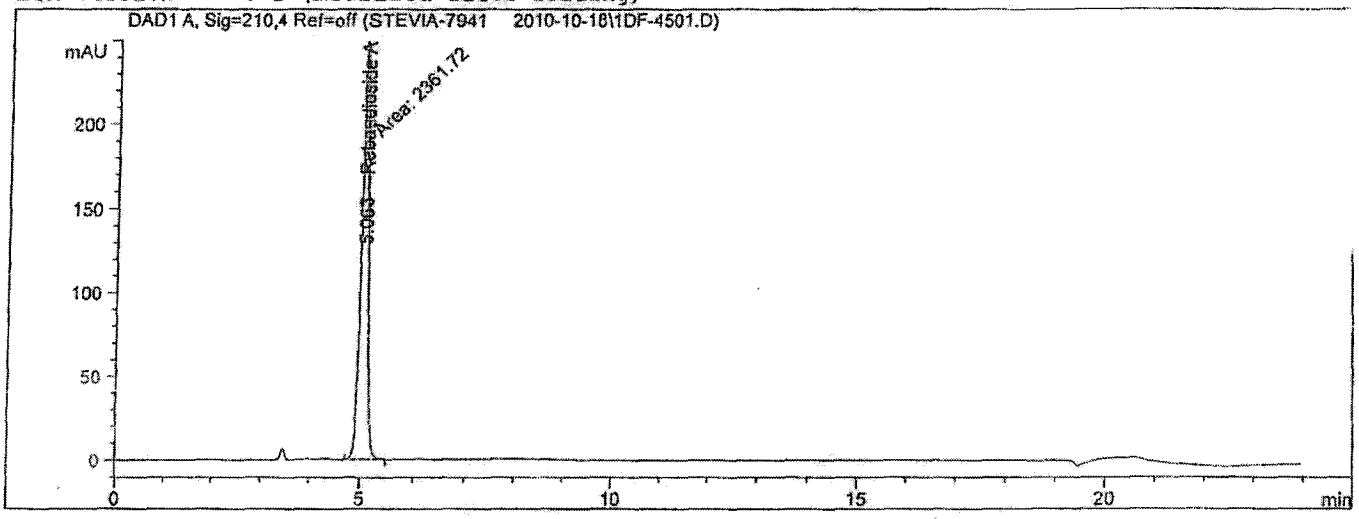
*** End of Report ***

000150

Sample Name: Rebaudioside Stk

```
Acq. Operator    : (b) (6)                    Seq. Line :  45
Acq. Instrument  : HPLC 10                     Location : P1-D-06
Injection Date   : 10/20/2010 2:26:04 AM            Inj :   1
                                              Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 12:07:46 PM by Mariel Esguerra
                   (modified after loading)
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1 (modified after loading)
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:07:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.063	MM	2361.71802	8.77661e-4	2.07279		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07279		

000151

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

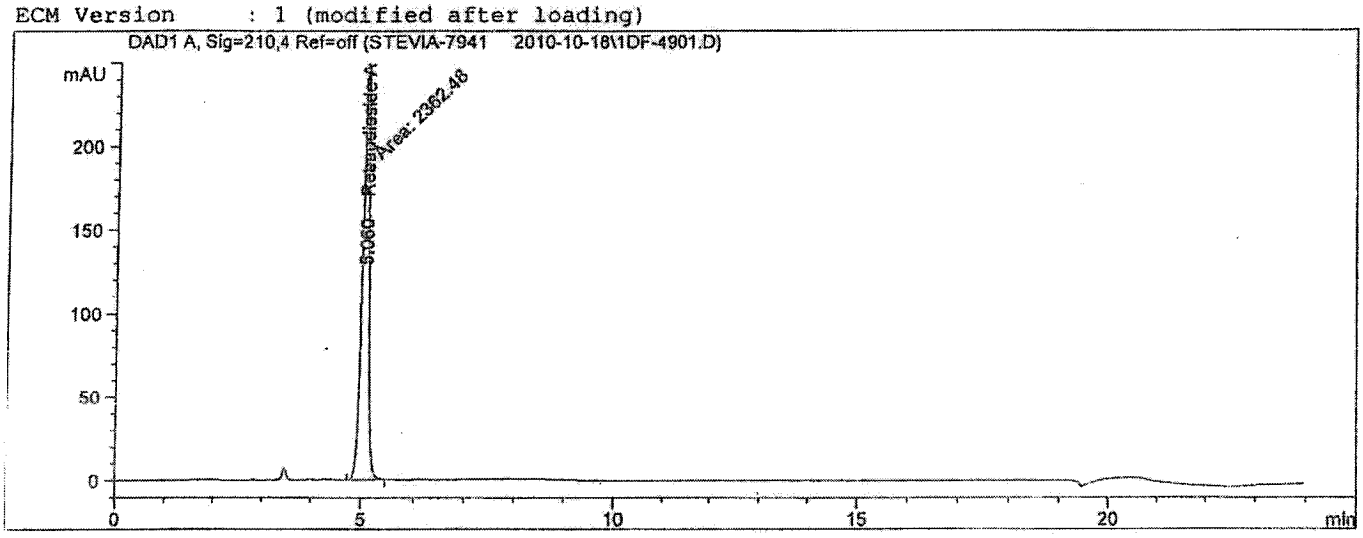
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

```
Acq. Operator   : (b) (6)                          Seq. Line :  49
Acq. Instrument : HPLC 10                          Location : P1-D-06
Injection Date  : 10/20/2010 5:04:59 AM                Inj :   1
                                                  Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 10/20/2010 12:07:46 PM by Mariel Esguerra
                  (modified after loading)
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version     : 1 (modified after loading)
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:07:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.060	MM	2362.47607	8.77661e-4	2.07345		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07345		

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

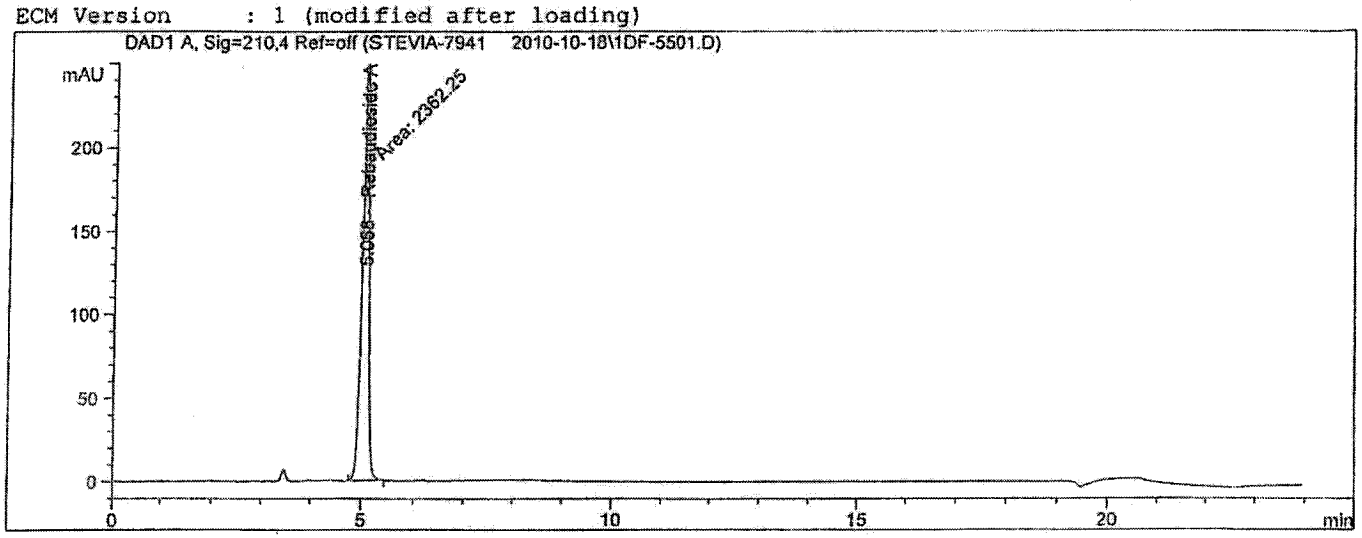
==========

*** End of Report ***

Sample Name: Rebaudioside Stk

```
Acq. Operator    : (b) (6)                          Seq. Line :  55
Acq. Instrument  : HPLC 10                           Location : P1-D-06
Injection Date   : 10/20/2010 9:03:26 AM                 Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 12:07:46 PM by Mariel Esguerra
                   (modified after loading)
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1 (modified after loading)
```



External Standard Report (Sample Amount is 0!)

```
Sorted By             :      Signal
Calib. Data Modified  :      Wednesday, October 20, 2010 12:07:45 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/mL]	Grp	Name
3.006		-	-	-		Steviolbioside
3.852		-	-	-		Rebaudioside B
4.143		-	-	-		Dulcoside A
4.466		-	-	-		Stevioside
4.918		-	-	-		Rebaudioside C
5.058	MM	2362.24634	8.77661e-4	2.07325		Rebaudioside A
5.800		-	-	-		Unknown
Totals :				2.07325		

Sample Name: Rebaudioside Stk

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

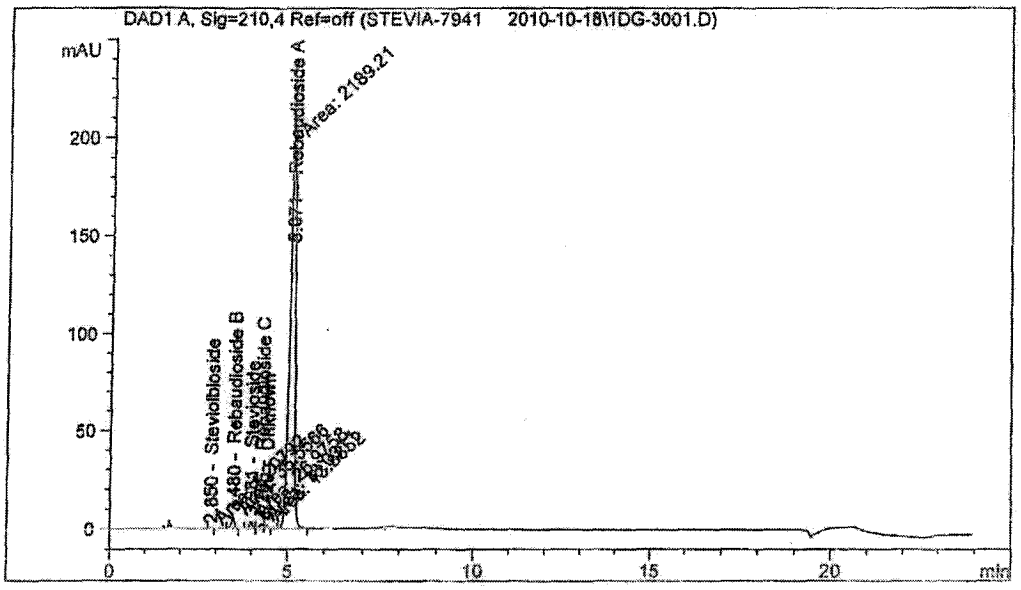
Appendix #7

Sample Suitability
(USP Tailing Factor)
(Theoretical Plates)

Sample Name: 10-7946A

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

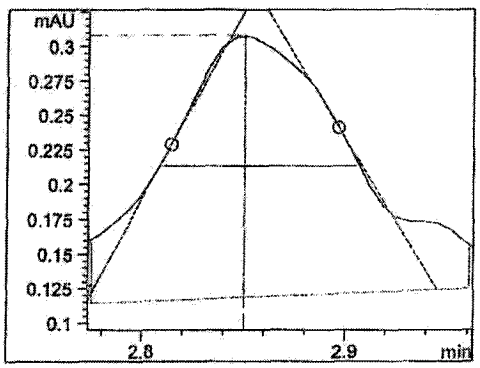


Sample Name: 10-7946A

Compound# 1 : Steviolbioside
Amount [%] : 0.0346

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	2.850	k':	-1.000
Height:	0.19	Area:	1.2
Start:	2.775	End:	2.961
Skew:	0.225	Excess:	-0.223
Width at half height:			0.096
5 sigma:			-1.000
tangent:			0.174
tailing:			-1.000
Symmetry:			0.723
USP Tailing:			-6.657
Integration type:			MF
Time increment [msec]:			400.0
Data points:			81



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	0.8			
M1:	2.861	Tangent method	4315	-1
M2:	0.001116	Halfwidth method	4883	-1
M3:	8.37378e-006	5 sigma method	-1	-1
M4:	3.45662e-006	Statistical	7337	-1

Relationship to preceeding peak:

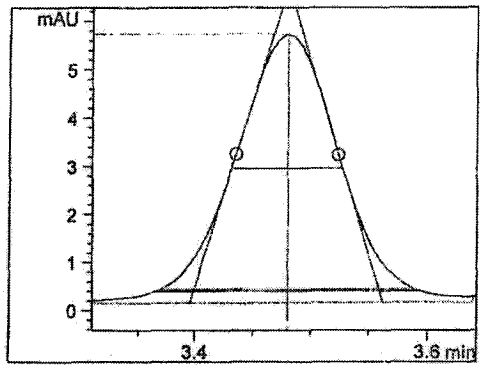
Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: 10-7946A

Compound# 3 : Rebaudioside B
Amount [%] : 1.2809

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	3.480	k':	-1.000
Height:	5.58	Area:	35.6
Start:	3.311	End:	3.639
Skew:	-0.086	Excess:	0.146
Width at half height:			0.095
5 sigma:			0.227
tangent:			0.167
tailing:			0.219
Symmetry:			1.032
USP Tailing:			0.987
Integration type:			MF
Time increment [msec]:			400.0
Data points:			84



Statistical moments (BB peak detection):

			Efficiency: Plates per ..	
			column	meter
M0:	33.7			
M1:	3.477	Tangent method	6941	-1
M2:	0.001763	Halfwidth method	7488	-1
M3:	-6.3412e-006	5 sigma method	5894	-1
M4:	9.77563e-006	Statistical	6859	-1

Relationship to preceeding peak:

		Selectivity:	1.221
Resolution Tangent method:	3.701	5 sigma method	-1.000
Halfwidth method	3.884	Statistical method	4.087

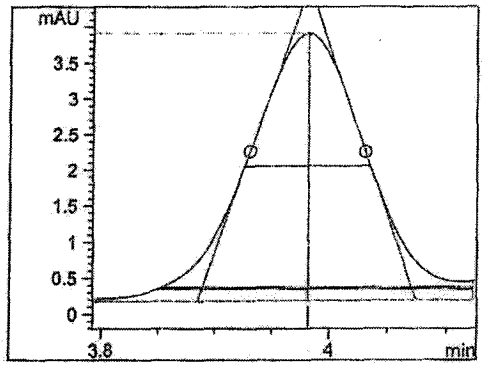
000160

Sample Name: 10-7946A

Compound# 4 : Stevioside
Amount [%] : 0.9718

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	3.981	k':	-1.000
Height:	3.73	Area:	27.0
Start:	3.795	End:	4.123
Skew:	-0.185	Excess:	-0.142
Width at half height:			0.109
5 sigma:			0.273
tangent:			0.190
tailing:			0.270
Symmetry:			1.026
USP Tailing:			1.051
Integration type:			MF
Time increment [msec]:			400.0
Data points:			62



Statistical moments (BB peak detection):

M0:	23.9
M1:	3.980
M2:	0.00197
M3:	-0.000016
M4:	0.000011

Efficiency: Plates per ..

	column	meter
Tangent method	7020	-1
Halfwidth method	7450	-1
5 sigma method	5305	-1
Statistical	8043	-1

Relationship to preceeding peak:

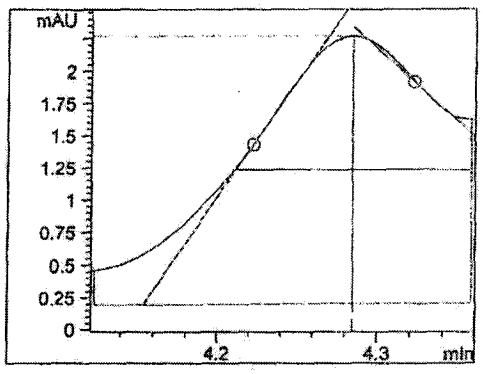
Selectivity:	1.144		
Resolution Tangent method:	2.806	5 sigma method	2.505
Halfwidth method	2.897	Statistical method	2.913

Sample Name: 10-7946A

Compound# 5 : Rebaudioside C
Amount [%] : 0.7669

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime: 4.285	k':	-1.000	
Height: 2.07	Area:	18.0	
Start: 4.123	End:	4.357	
Skew: -0.249	Excess:	-0.459	
Width at half height:		0.146	
5 sigma:		-1.000	
tangent:		0.320	
tailing:		-1.000	
Symmetry:		1.286	
USP Tailing:		-3.093	
Integration type:		MF	
Time increment [msec]:		400.0	
Data points:		41	



Statistical moments (BB peak detection):

M0:	6.1
M1:	4.266
M2:	0.001372
M3:	-0.000013
M4:	4.78189e-006

Efficiency: Plates per ..

	column	meter
Tangent method	2874	-1
Halfwidth method	4744	-1
5 sigma method	-1	-1
Statistical	13267	-1

Relationship to preceeding peak:

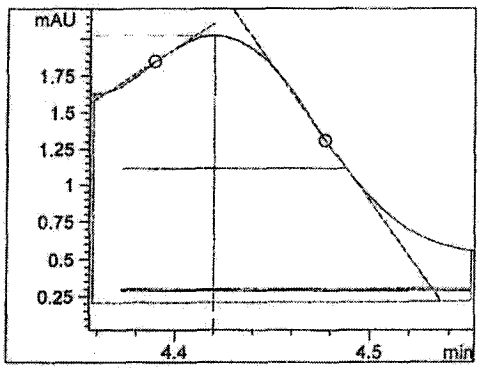
Resolution Tangent method:	1.190	Selectivity: 5 sigma method	1.076 -1.000
Halfwidth method	1.397	Statistical method	1.756

Sample Name: 10-7946A

Compound# 6 : Unknown
Amount [%] : 0.6026

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.420	k':	-1.000
Height:	1.81	Area:	13.9
Start:	4.357	End:	4.551
Skew:	0.051	Excess:	-0.658
Width at half height:			0.115
5 sigma:			0.178
tangent:			0.344
tailing:			0.178
Symmetry:			0.715
USP Tailing:			1.876
Integration type:			MF
Time increment [msec]:			400.0
Data points:			60



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	3.9			
M1:	4.431	Tangent method	2641	-1
M2:	0.000891	Halfwidth method	8183	-1
M3:	1.36629e-006	5 sigma method	15357	-1
M4:	1.86066e-006	Statistical	22027	-1

Relationship to preceeding peak:

Selectivity:	1.032		
Resolution Tangent method:	0.407	5 sigma method	-1.000
Halfwidth method	0.608	Statistical method	1.231

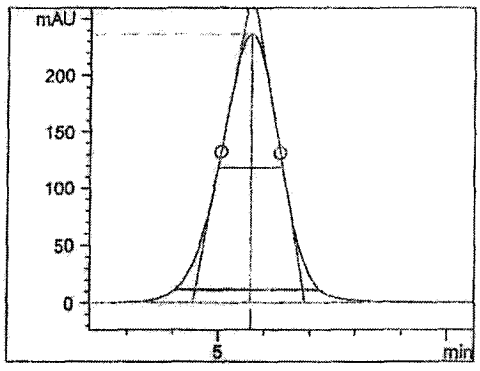
000163

Sample Name: 10-7946A

Compound# 7 : Rebaudioside A
Amount [%] : 95.0169

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.071	k':	-1.000
Height:	236.22	Area:	2189.2
Start:	4.722	End:	5.553
Skew:	0.158	Excess:	1.487
Width at half height:			0.140
5 sigma:			0.322
tangent:			0.244
tailing:			0.313
Symmetry:			1.054
USP Tailing:			0.958
Integration type:			FM
Time increment [msec]:			400.0
Data points:			126



Statistical moments (BB peak detection):

M0:	2182.5
M1:	5.065
M2:	0.004479
M3:	0.000047
M4:	0.00009

Efficiency: Plates per ..

	column	meter
Tangent method	6925	-1
Halfwidth method	7268	-1
5 sigma method	6213	-1
Statistical	5727	-1

Relationship to preceeding peak:

Selectivity:	1.147		
Resolution Tangent method:	2.215	5 sigma method	3.255
Halfwidth method	3.000	Statistical method	3.275

Sample Name: 10-7946A

#	Ret.Time [min]	Amount [%]	Name	Page #
1	2.850	0.0346	Steviolbioside	3
2	3.480	1.2809	Rebaudioside B	4
3	3.981	0.9718	Stevioside	5
4	4.285	0.7669	Rebaudioside C	6
5	4.420	0.6026	Unknown	7
6	5.071	95.0169	Rebaudioside A	8
	Total:	98.6738		

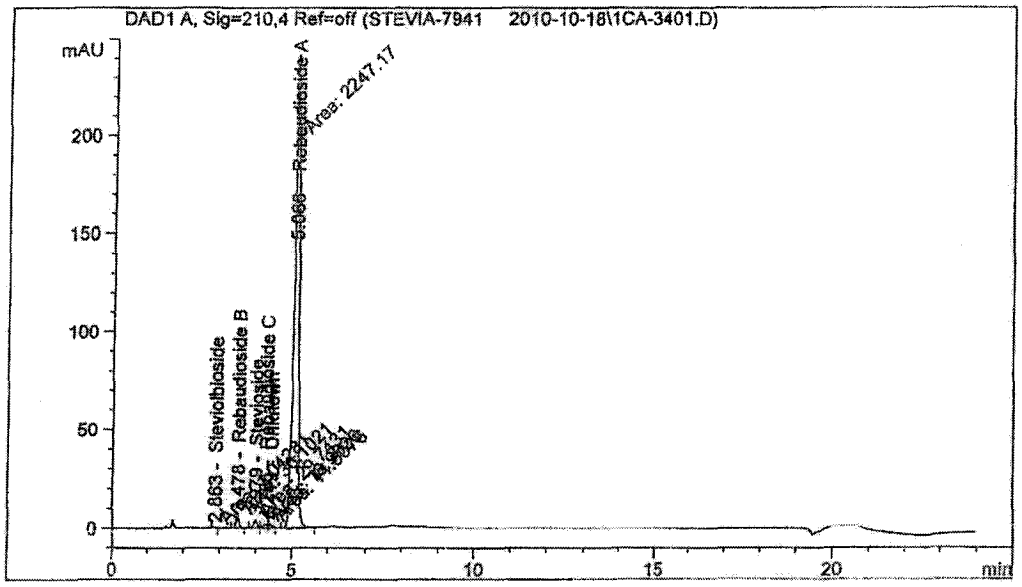
*** End of Report ***

000165

Sample Name: 10-7947A

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off



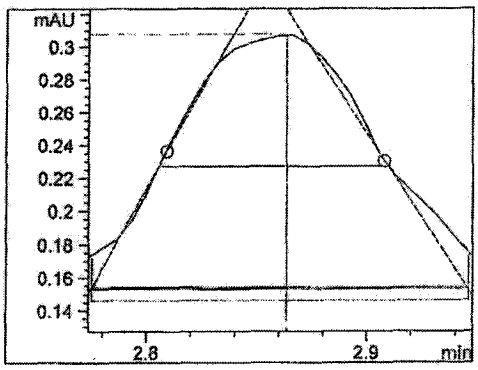
000166

Sample Name: 10-7947A

Compound# 1 : Steviolbioside
Amount [%] : 0.0300

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	2.863	k':	-1.000
Height:	0.16	Area:	1.0
Start:	2.775	End:	2.946
Skew:	0.097	Excess:	-0.618
Width at half height:			0.104
5 sigma:			0.171
tangent:			0.178
tailing:			0.171
Symmetry:			1.133
USP Tailing:			0.965
Integration type:			MM
Time increment [msec]:			400.0
Data points:			83



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	0.8			
M1:	2.861	Tangent method	4162	-1
M2:	0.001133	Halfwidth method	4200	-1
M3:	3.69686e-006	5 sigma method	6985	-1
M4:	3.05906e-006	Statistical	7225	-1

Relationship to preceeding peak:

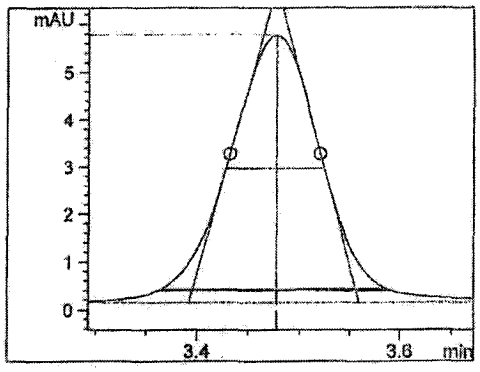
Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: 10-7947A

Compound# 3 : Rebaudioside B
Amount [%] : 1.3065

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	3.478	k':	-1.000
Height:	5.64	Area:	36.1
Start:	3.296	End:	3.668
Skew:	0.015	Excess:	0.495
Width at half height:			0.095
5 sigma:			0.229
tangent:			0.167
tailing:			0.221
Symmetry:			1.015
USP Tailing:			0.994
Integration type:			MF
Time increment [msec]:			400.0
Data points:			82



Statistical moments (BB peak detection):

M0:	34.9
M1:	3.480
M2:	0.001944
M3:	1.24429e-006
M4:	0.000013

Efficiency: Plates per ..

	column	meter
Tangent method	6956	-1
Halfwidth method	7476	-1
5 sigma method	5748	-1
Statistical	6228	-1

Relationship to preceeding peak:

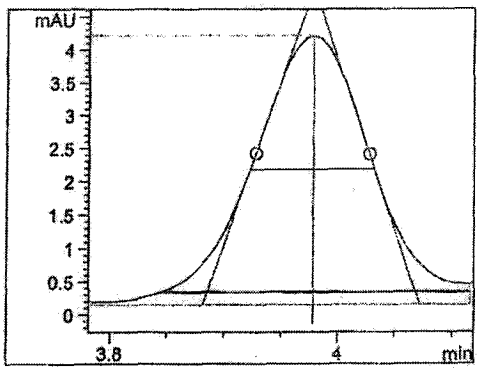
Selectivity:	1.214		
Resolution Tangent method:	3.568	5 sigma method	3.832
Halfwidth method	3.632	Statistical method	3.976

Sample Name: 10-7947A

Compound# 4 : Stevioside
Amount [%] : 1.0763

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	3.979	k':	-1.000
Height:	4.07	Area:	29.7
Start:	3.784	End:	4.115
Skew:	-0.191	Excess:	-0.069
Width at half height:			0.109
5 sigma:			0.273
tangent:			0.191
tailing:			0.269
Symmetry:			1.044
USP Tailing:			1.014
Integration type:			MF
Time increment [msec]:			400.0
Data points:			62



Statistical moments (BB peak detection):

M0:	26.1
M1:	3.972
M2:	0.002021
M3:	-0.000017
M4:	0.000012

Efficiency: Plates per ..

	column	meter
Tangent method	6969	-1
Halfwidth method	7440	-1
5 sigma method	5311	-1
Statistical	7805	-1

Relationship to preceeding peak:

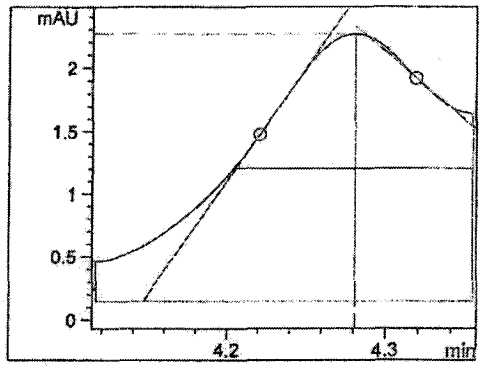
Selectivity:	1.144		
Resolution Tangent method:	2.805	5 sigma method	2.495
Halfwidth method	2.898	Statistical method	2.766

Sample Name: 10-7947A

Compound# 5 : Rebaudioside C
Amount [%] : 0.8102

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.281	k':	-1.000
Height:	2.12	Area:	19.0
Start:	4.115	End:	4.354
Skew:	-0.232	Excess:	-0.476
Width at half height:			0.149
5 sigma:			-1.000
tangent:			0.336
tailing:			-1.000
Symmetry:			1.325
USP Tailing:			-3.021
Integration type:			MF
Time increment [msec]:			400.0
Data points:			41



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	5.8			
M1:	4.268	Tangent method	2604	-1
M2:	0.001316	Halfwidth method	4553	-1
M3:	-0.000011	5 sigma method	-1	-1
M4:	4.37399e-006	Statistical	13839	-1

Relationship to preceeding peak:

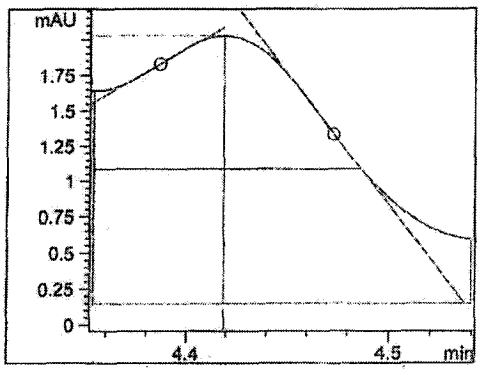
Selectivity:	1.076		
Resolution Tangent method:	1.148	5 sigma method	-1.000
Halfwidth method	1.376	Statistical method	1.822

Sample Name: 10-7947A

Compound# 6 : Unknown
Amount [%] : 0.6325

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.419	k':	-1.000
Height:	1.88	Area:	14.5
Start:	4.354	End:	4.540
Skew:	0.044	Excess:	-0.603
Width at half height:			0.132
5 sigma:			-1.000
tangent:			0.360
tailing:			-1.000
Symmetry:			0.764
USP Tailing:			-7.675
Integration type:			MF
Time increment [msec]:			400.0
Data points:			56



Statistical moments (BB peak detection):

M0:	3.9		
M1:	4.431	Tangent method	2417
M2:	0.000922	Halfwidth method	6173
M3:	1.22202e-006	5 sigma method	-1
M4:	2.03818e-006	Statistical	21291

Efficiency: Plates per ..

column	meter
-1	-1
-1	-1
-1	-1
-1	-1

Relationship to preceeding peak:

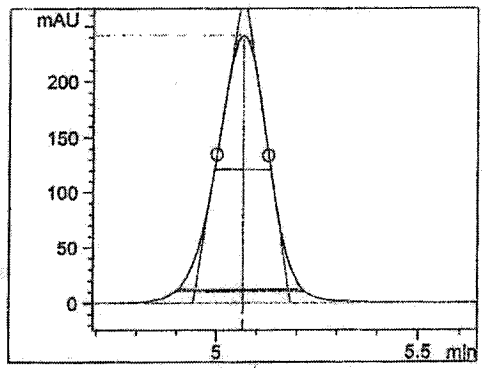
Resolution Tangent method:	0.397	Selectivity:	1.032
Halfwidth method	0.575	5 sigma method	-1.000
		Statistical method	1.221

Sample Name: 10-7947A

Compound# 7 : Rebaudioside A
Amount [%] : 97.9773

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.066	k':	-1.000
Height:	241.84	Area:	2247.2
Start:	4.700	End:	5.635
Skew:	0.303	Excess:	2.286
Width at half height:			0.138
5 sigma:			0.320
tangent:			0.244
tailing:			0.311
Symmetry:			1.050
USP Tailing:			0.958
Integration type:			FM
Time increment [msec]:			400.0
Data points:			142



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2235.2			
M1:	5.065	Tangent method	6917	-1
M2:	0.004604	Halfwidth method	7491	-1
M3:	0.000095	5 sigma method	6267	-1
M4:	0.000112	Statistical	5572	-1

Relationship to preceeding peak:

Selectivity:	1.147		
Resolution Tangent method:	2.147	5 sigma method	-1.000
Halfwidth method	2.816	Statistical method	3.226

Sample Name: 10-7947A

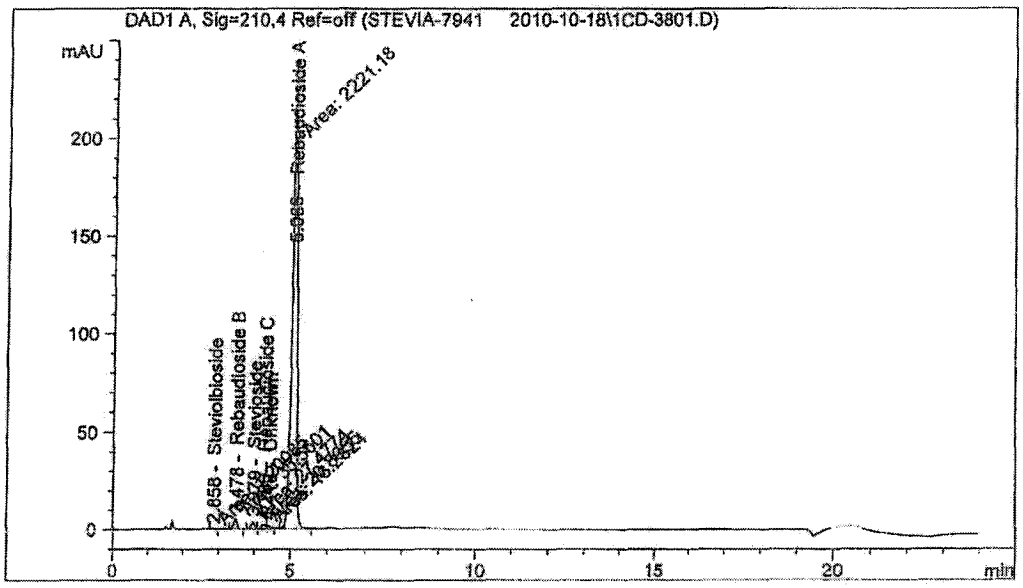
#	Ret.Time [min]	Amount [%]	Name	Page #
1	2.863	0.0300	Steviolbioside	3
2	3.478	1.3065	Rebaudioside B	4
3	3.979	1.0763	Stevioside	5
4	4.281	0.8102	Rebaudioside C	6
5	4.419	0.6325	Unknown	7
6	5.066	97.9773	Rebaudioside A	8
	Total:	101.8329		

*** End of Report ***

Sample Name: 10-7948A

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off



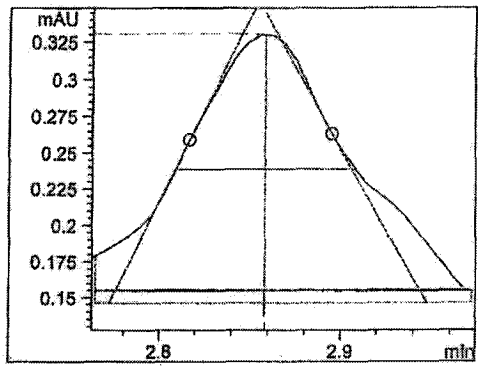
000174

Sample Name: 10-7948A

Compound# 1 : Steviolbioside
Amount [%] : 0.0348

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	2.858	k':	-1.000
Height:	0.18	Area:	1.2
Start:	2.763	End:	2.972
Skew:	0.174	Excess:	-0.443
Width at half height:			0.097
5 sigma:			0.208
tangent:			0.176
tailing:			0.208
Symmetry:			0.925
USP Tailing:			1.097
Integration type:			MM
Time increment [msec]:			400.0
Data points:			83



Statistical moments (BB peak detection):

M0:	1.0
M1:	2.866
M2:	0.001531
M3:	0.00001
M4:	5.99164e-006

Efficiency: Plates per ..

	column	meter
Tangent method	4231	-1
Halfwidth method	4778	-1
5 sigma method	4721	-1
Statistical	5367	-1

Relationship to preceeding peak:

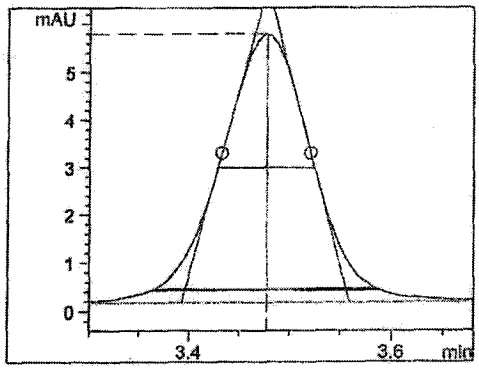
Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: 10-7948A

Compound# 3 : Rebaudioside B
Amount [%] : 1.2956

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	3.478	k':	-1.000
Height:	5.61	Area:	35.6
Start:	3.303	End:	3.676
Skew:	0.019	Excess:	0.539
Width at half height:			0.093
5 sigma:			0.224
tangent:			0.166
tailing:			0.217
Symmetry:			1.030
USP Tailing:			0.986
Integration type:			MF
Time increment [msec]:			400.0
Data points:			83



Statistical moments (BB peak detection):

			Efficiency: Plates per ..	
			column	meter
M0:	34.8			
M1:	3.478	Tangent method	7036	-1
M2:	0.001941	Halfwidth method	7692	-1
M3:	1.64378e-006	5 sigma method	6027	-1
M4:	0.000013	Statistical	6232	-1

Relationship to preceeding peak:

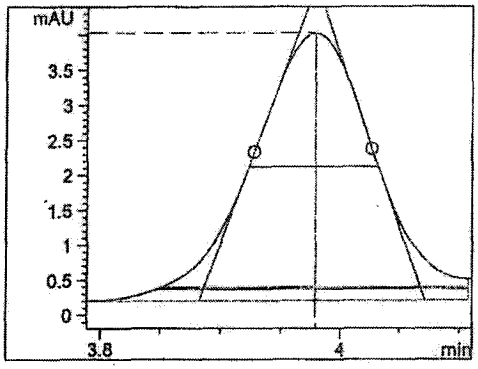
		Selectivity:	1.217
Resolution Tangent method:	3.626	5 sigma method	3.585
Halfwidth method	3.817	Statistical method	3.676

Sample Name: 10-7948A

Compound# 4 : Stevioside
Amount [%] : 1.0011

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	3.979	k':	-1.000
Height:	3.83	Area:	27.5
Start:	3.791	End:	4.108
Skew:	-0.195	Excess:	-0.094
Width at half height:			0.108
5 sigma:			0.259
tangent:			0.189
tailing:			0.256
Symmetry:			1.034
USP Tailing:			1.006
Integration type:			MF
Time increment [msec]:			400.0
Data points:			59



Statistical moments (BB peak detection):

M0:	24.5
M1:	3.977
M2:	0.001981
M3:	-0.000017
M4:	0.000011

Efficiency: Plates per ..

	column	meter
Tangent method	7067	-1
Halfwidth method	7574	-1
5 sigma method	5906	-1
Statistical	7984	-1

Relationship to preceeding peak:

Selectivity:	1.144		
Resolution Tangent method:	2.823	5 sigma method	2.595
Halfwidth method	2.931	Statistical method	2.819

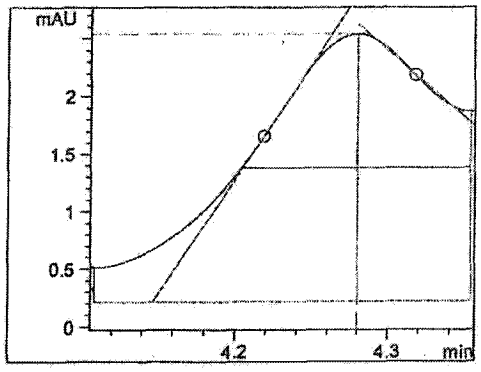
000177

Sample Name: 10-7948A

Compound# 5 : Rebaudioside C
Amount [%] : 0.8989

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.280	k':	-1.000
Height:	2.32	Area:	20.9
Start:	4.108	End:	4.354
Skew:	-0.177	Excess:	-0.515
Width at half height:			0.149
5 sigma:			-1.000
tangent:			0.340
tailing:			-1.000
Symmetry:			1.311
USP Tailing:			-2.906
Integration type:			MF
Time increment [msec]:			400.0
Data points:			43



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	6.6			
M1:	4.264	Tangent method	2539	-1
M2:	0.00134	Halfwidth method	4579	-1
M3:	-8.69182e-00	5 sigma method	-1	-1
M4:	4.46421e-006	Statistical	13568	-1

Relationship to preceeding peak:

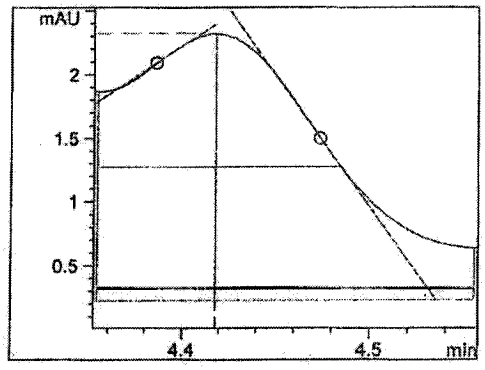
Selectivity:	1.076		
Resolution Tangent method:	1.138	5 sigma method	-1.000
Halfwidth method	1.380	Statistical method	1.771

Sample Name: 10-7948A

Compound# 6 : Unknown
Amount [%] : 0.7148

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.418	k':	-1.000
Height:	2.10	Area:	16.3
Start:	4.354	End:	4.555
Skew:	0.040	Excess:	-0.645
Width at half height:			0.130
5 sigma:			0.201
tangent:			0.348
tailing:			0.201
Symmetry:			0.729
USP Tailing:			1.562
Integration type:			MF
Time increment [msec]:			400.0
Data points:			59



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	4.2			
M1:	4.427	Tangent method	2586	-1
M2:	0.000854	Halfwidth method	6352	-1
M3:	1.00108e-006	5 sigma method	12084	-1
M4:	1.71702e-006	Statistical	22952	-1

Relationship to preceeding peak:

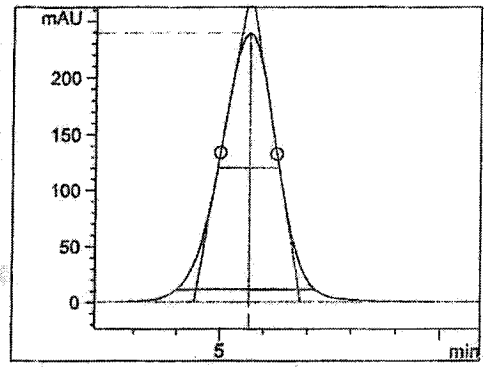
Selectivity:	1.032		
Resolution Tangent method:	0.401	5 sigma method	-1.000
Halfwidth method	0.580	Statistical method	1.234

Sample Name: 10-7948A

Compound# 7 : Rebaudioside A
Amount [%] : 97.5051

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.066	k':	-1.000
Height:	239.37	Area:	2221.2
Start:	4.715	End:	5.583
Skew:	0.238	Excess:	1.881
Width at half height:			0.138
5 sigma:			0.322
tangent:			0.244
tailing:			0.313
Symmetry:			1.053
USP Tailing:			0.956
Integration type:			FM
Time increment [msec]:			400.0
Data points:			132



Statistical moments (BB peak detection):

M0:	2213.3
M1:	5.063
M2:	0.004554
M3:	0.000073
M4:	0.000101

Efficiency: Plates per ..

	column	meter
Tangent method	6911	-1
Halfwidth method	7491	-1
5 sigma method	6180	-1
Statistical	5630	-1

Relationship to preceeding peak:

Selectivity:	1.147		
Resolution Tangent method:	2.192	5 sigma method	3.097
Halfwidth method	2.839	Statistical method	3.289

Sample Name: 10-7948A

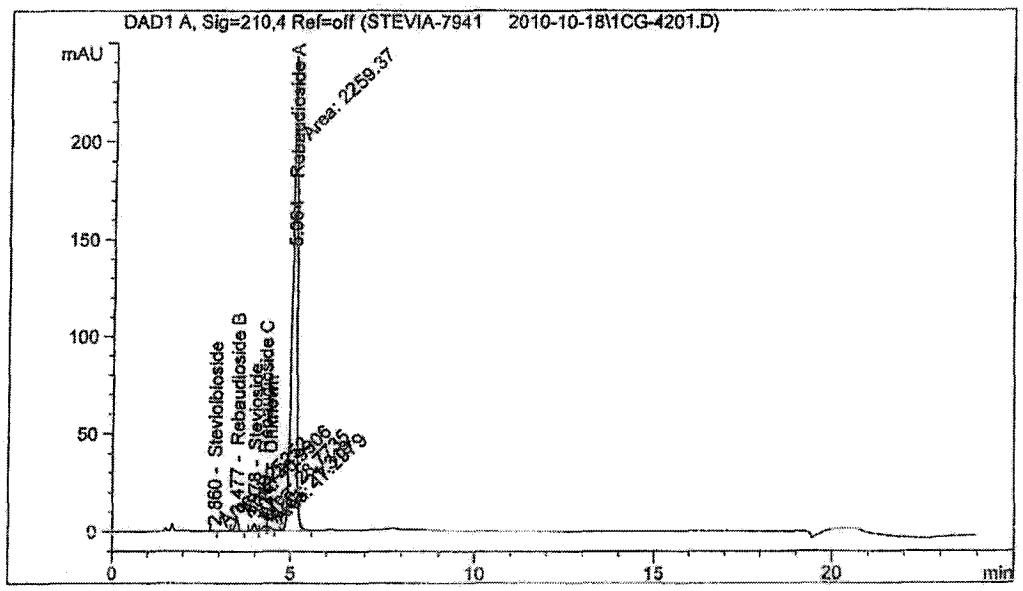
#	Ret.Time [min]	Amount [%]	Name	Page #
1	2.858	0.0348	Steviolbioside	3
2	3.478	1.2956	Rebaudioside B	4
3	3.979	1.0011	Stevioside	5
4	4.280	0.8989	Rebaudioside C	6
5	4.418	0.7148	Unknown	7
6	5.066	97.5051	Rebaudioside A	8
	Total:	101.4503		

*** End of Report ***

Sample Name: 10-7949A

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off



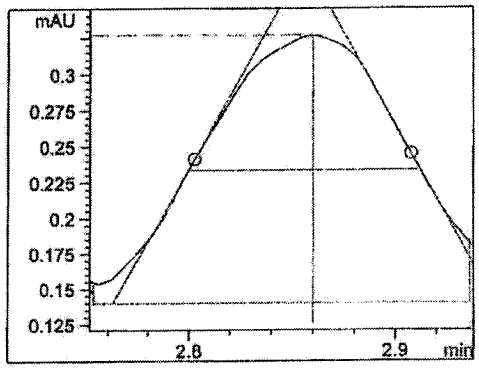
000182

Sample Name: 10-7949A

Compound# 1 : Steviolbioside
Amount [%] : 0.0357

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	2.860	k':	-1.000
Height:	0.19	Area:	1.3
Start:	2.752	End:	2.937
Skew:	-0.099	Excess:	-0.672
Width at half height:			0.111
5 sigma:			-1.000
tangent:			0.186
tailing:			-1.000
Symmetry:			1.158
USP Tailing:			-4.652
Integration type:			MM
Time increment [msec]:			400.0
Data points:			86



Statistical moments (BB peak detection):

			Efficiency: Plates per ..	
			column	meter
M0:	0.9			
M1:	2.849	Tangent method	3771	-1
M2:	0.001228	Halfwidth method	3700	-1
M3:	-4.2492e-006	5 sigma method	-1	-1
M4:	3.50957e-006	Statistical	6610	-1

Relationship to preceeding peak:

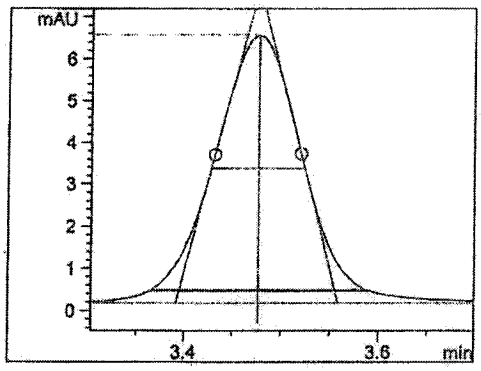
		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: 10-7949A

Compound# 3 : Rebaudioside B
Amount [%] : 1.4687

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	3.477	k':	-1.000
Height:	6.40	Area:	41.0
Start:	3.305	End:	3.695
Skew:	0.221	Excess:	0.858
Width at half height:			0.095
5 sigma:			0.227
tangent:			0.167
tailing:			0.220
Symmetry:			1.003
USP Tailing:			1.005
Integration type:			MF
Time increment [msec]:			400.0
Data points:			82



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	39.8			
M1:	3.474	Tangent method	6948	-1
M2:	0.002019	Halfwidth method	7472	-1
M3:	0.00002	5 sigma method	5882	-1
M4:	0.000016	Statistical	5978	-1

Relationship to preceeding peak:

		Selectivity:	1.216
Resolution Tangent method:	3.493	5 sigma method	-1.000
Halfwidth method	3.529	Statistical method	3.905

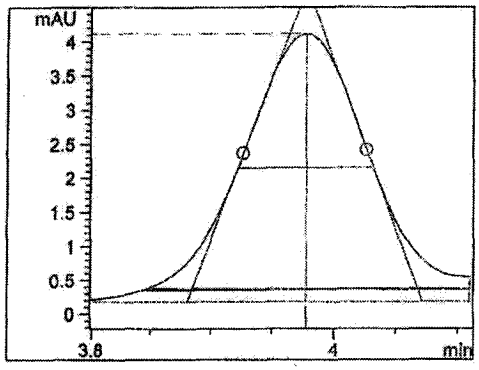
000184

Sample Name: 10-7949A

Compound# 4 : Stevioside
Amount [%] : 1.0310

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	3.978	k':	-1.000
Height:	3.94	Area:	28.8
Start:	3.802	End:	4.110
Skew:	-0.192	Excess:	-0.095
Width at half height:			0.109
5 sigma:			0.266
tangent:			0.191
tailing:			0.263
Symmetry:			1.009
USP Tailing:			1.009
Integration type:			MF
Time increment [msec]:			400.0
Data points:			61



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	24.8			
M1:	3.974	Tangent method	6920	-1
M2:	0.001973	Halfwidth method	7436	-1
M3:	-0.000017	5 sigma method	5571	-1
M4:	0.000011	Statistical	8004	-1

Relationship to preceeding peak:

		Selectivity:	1.144
Resolution Tangent method:	2.798	5 sigma method	2.540
Halfwidth method	2.897	Statistical method	2.798

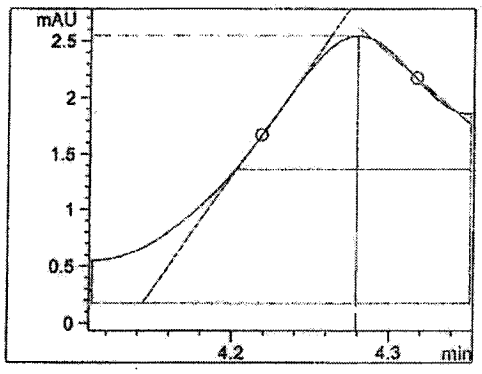
000185

Sample Name: 10-7949A

Compound# 5 : Rebaudioside C
Amount [%] : 0.9019

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.279	k':	-1.000
Height:	2.37	Area:	21.3
Start:	4.110	End:	4.351
Skew:	-0.208	Excess:	-0.535
Width at half height:			0.149
5 sigma:			-1.000
tangent:			0.342
tailing:			-1.000
Symmetry:			1.347
USP Tailing:			-2.965
Integration type:			MF
Time increment [msec]:			400.0
Data points:			42



Statistical moments (BB peak detection):

M0:	6.2
M1:	4.266
M2:	0.001249
M3:	-9.19384e-00
M4:	3.84315e-006

Efficiency: Plates per ..

	column	meter
Tangent method	2506	-1
Halfwidth method	4549	-1
5 sigma method	-1	-1
Statistical	14575	-1

Relationship to preceeding peak:

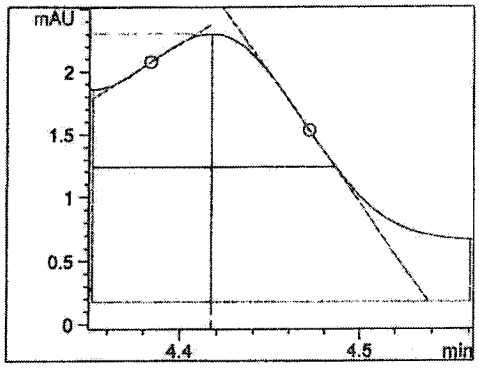
Resolution Tangent method:	1.131	Selectivity:	1.076
Halfwidth method	1.373	5 sigma method	1.000
		Statistical method	1.833

Sample Name: 10-7949A

Compound# 6 : Unknown
Amount [%] : 0.7467

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.417	k':	-1.000
Height:	2.13	Area:	17.3
Start:	4.351	End:	4.560
Skew:	-0.001	Excess:	-0.638
Width at half height:			0.134
5 sigma:			-1.000
tangent:			0.363
tailing:			-1.000
Symmetry:			0.713
USP Tailing:			-7.601
Integration type:			MF
Time increment [msec]:			400.0
Data points:			58



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	4.2			
M1:	4.428	Tangent method	2364	-1
M2:	0.000885	Halfwidth method	5994	-1
M3:	-2.83056e-00	5 sigma method	-1	-1
M4:	1.85172e-006	Statistical	22150	-1

Relationship to preceeding peak:

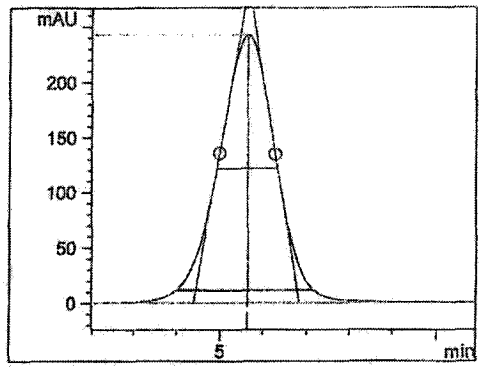
Selectivity:	1.032		
Resolution Tangent method:	0.391	5 sigma method	-1.000
Halfwidth method	0.572	Statistical method	1.247

Sample Name: 10-7949A

Compound# 7 : Rebaudioside A
Amount [%] : 97.5355

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	5.064	k':	-1.000
Height:	243.08	Area:	2259.4
Start:	4.706	End:	5.582
Skew:	0.275	Excess:	2.086
Width at half height:			0.138
5 sigma:			0.322
tangent:			0.244
tailing:			0.311
Symmetry:			1.049
USP Tailing:			0.959
Integration type:			FM
Time increment [msec]:			400.0
Data points:			133



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2245.7			
M1:	5.059	Tangent method	6905	-1
M2:	0.004573	Halfwidth method	7484	-1
M3:	0.000085	5 sigma method	6175	-1
M4:	0.000106	Statistical	5597	-1

Relationship to preceeding peak:

		Selectivity:	1.146
Resolution Tangent method:	2.131	5 sigma method	-1.000
Halfwidth method	2.794	Statistical method	3.238

000188

Sample Name: 10-7949A

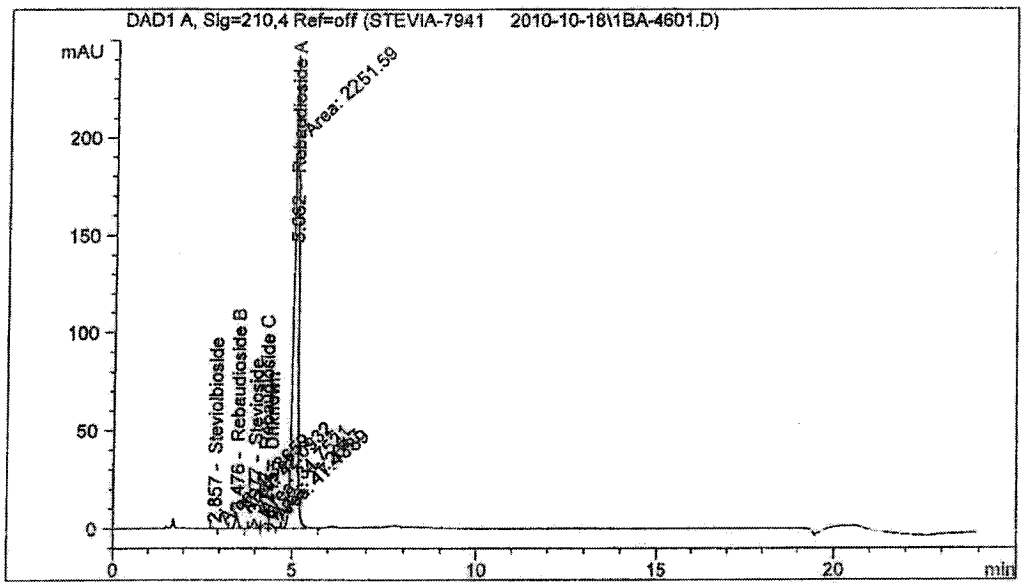
#	Ret.Time [min]	Amount [%]	Name	Page #
1	2.860	0.0357	Steviolbioside	3
2	3.477	1.4687	Rebaudioside B	4
3	3.978	1.0310	Stevioside	5
4	4.279	0.9019	Rebaudioside C	6
5	4.417	0.7467	Unknown	7
6	5.064	97.5355	Rebaudioside A	8
	Total:	101.7195		

*** End of Report ***

Sample Name: 10-7950A

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

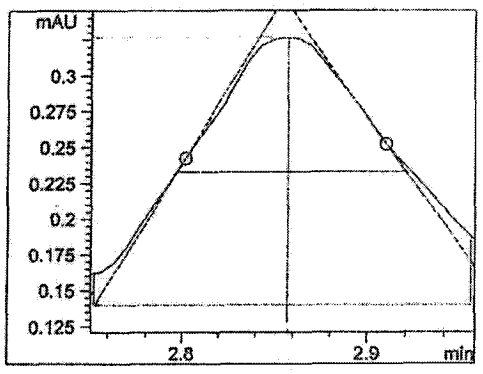


Sample Name: 10-7950A

Compound# 1 : Steviolbioside
Amount [%] : 0.0396

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	2.857	k':	-1.000
Height:	0.19	Area:	1.4
Start:	2.752	End:	2.954
Skew:	0.019	Excess:	-0.600
Width at half height:			0.124
5 sigma:			-1.000
tangent:			0.218
tailing:			-1.000
Symmetry:			0.940
USP Tailing:			-4.777
Integration type:			MM
Time increment [msec]:			400.0
Data points:			95



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	1.0			
M1:	2.858	Tangent method	2761	-1
M2:	0.001597	Halfwidth method	2942	-1
M3:	1.22395e-006	5 sigma method	-1	-1
M4:	6.12546e-006	Statistical	5115	-1

Relationship to preceeding peak:

Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

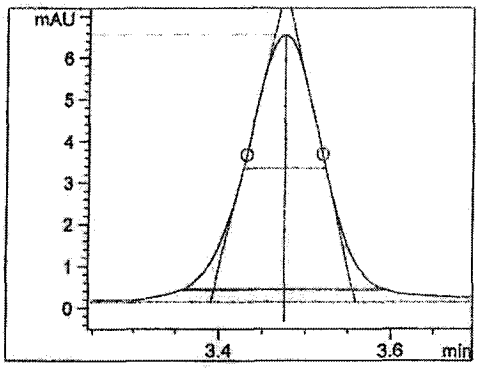
000191

Sample Name: 10-7950A

Compound# 3 : Rebaudioside B
Amount [%] : 1.5119

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	3.476	k':	-1.000
Height:	6.43	Area:	42.1
Start:	3.249	End:	3.692
Skew:	0.062	Excess:	0.730
Width at half height:			0.095
5 sigma:			0.240
tangent:			0.168
tailing:			0.229
Symmetry:			1.007
USP Tailing:			0.997
Integration type:			MF
Time increment [msec]:			400.0
Data points:			86



Statistical moments (BB peak detection):

M0:	39.8
M1:	3.473
M2:	0.002028
M3:	5.61762e-006
M4:	0.000015

Efficiency: Plates per ..

	column	meter
Tangent method	6840	-1
Halfwidth method	7468	-1
5 sigma method	5243	-1
Statistical	5950	-1

Relationship to preceeding peak:

Resolution Tangent method:	3.207	Selectivity: 5 sigma method	1.216 -1.000
Halfwidth method	3.323	Statistical method	3.617

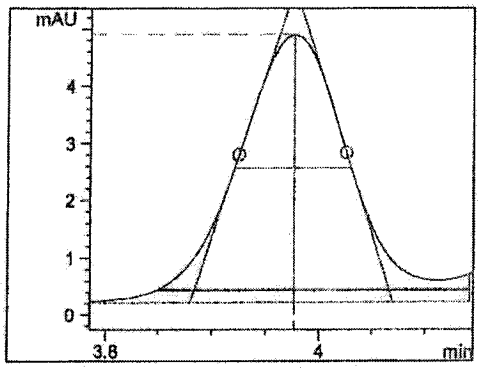
000192

Sample Name: 10-7950A

Compound# 4 : Stevioside
Amount [%] : 1.2482

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	3.977	k':	-1.000
Height:	4.69	Area:	34.8
Start:	3.788	End:	4.142
Skew:	-0.202	Excess:	-0.137
Width at half height:			0.108
5 sigma:			0.294
tangent:			0.190
tailing:			0.291
Symmetry:			0.000
USP Tailing:			1.157
Integration type:			MF
Time increment [msec]:			400.0
Data points:			66



Statistical moments (BB peak detection):

M0:	29.3
M1:	3.970
M2:	0.001903
M3:	-0.000017
M4:	0.00001

Efficiency: Plates per ..

	column	meter
Tangent method	6983	-1
Halfwidth method	7543	-1
5 sigma method	4564	-1
Statistical	8283	-1

Relationship to preceeding peak:

Selectivity:	1.144		
Resolution Tangent method:	2.796	5 sigma method	2.345
Halfwidth method	2.909	Statistical method	2.799

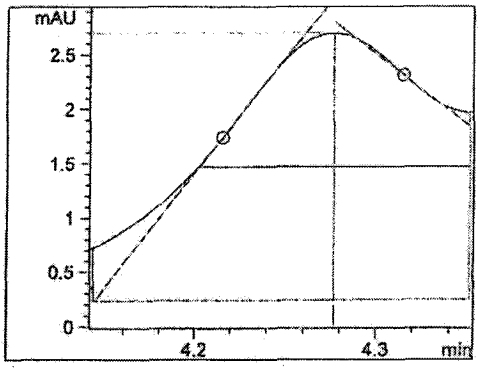
000193

Sample Name: 10-7950A

Compound# 5 : Rebaudioside C
Amount [%] : 0.9018

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	4.277	k':	-1.000
Height:	2.46	Area:	21.3
Start:	4.142	End:	4.351
Skew:	-0.278	Excess:	-0.396
Width at half height:			0.149
5 sigma:			-1.000
tangent:			0.332
tailing:			-1.000
Symmetry:			1.199
USP Tailing:			-3.712
Integration type:			FM
Time increment [msec]:			400.0
Data points:			38



Statistical moments (BB peak detection):

M0:	7.1
M1:	4.260
M2:	0.00142
M3:	-0.000015
M4:	5.25496e-006

Efficiency: Plates per ..

	column	meter
Tangent method	2660	-1
Halfwidth method	4559	-1
5 sigma method	-1	-1
Statistical	12774	-1

Relationship to preceeding peak:

Resolution Tangent method:	1.149	Selectivity:	1.075
Halfwidth method	1.372	5 sigma method	-1.000
		Statistical method	1.784

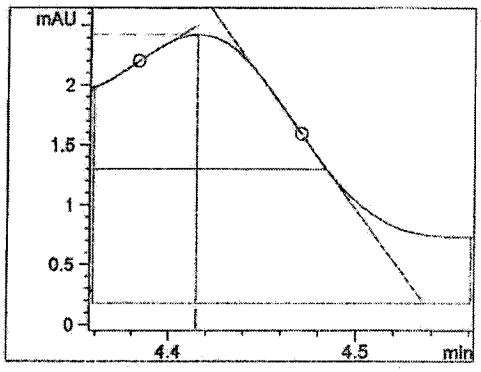
000194

Sample Name: 10-7950A

Compound# 6 : Unknown
Amount [%] : 0.7544

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	4.415	k':	-1.000
Height:	2.25	Area:	17.4
Start:	4.358	End:	4.560
Skew:	0.000	Excess:	-0.698
Width at half height:			0.125
5 sigma:			-1.000
tangent:			0.365
tailing:			-1.000
Symmetry:			0.605
USP Tailing:			-8.890
Integration type:			FM
Time increment [msec]:			400.0
Data points:			58



Statistical moments (BB peak detection):

M0:	3.9
M1:	4.426
M2:	0.000752
M3:	6.56188e-009
M4:	1.29991e-006

Efficiency: Plates per ..

	column	meter
Tangent method	2344	-1
Halfwidth method	6937	-1
5 sigma method	-1	-1
Statistical	26069	-1

Relationship to preceeding peak:

Resolution Tangent method:	0.396	Selectivity:	1.032
Halfwidth method	0.592	5 sigma method	-1.000
		Statistical method	1.278

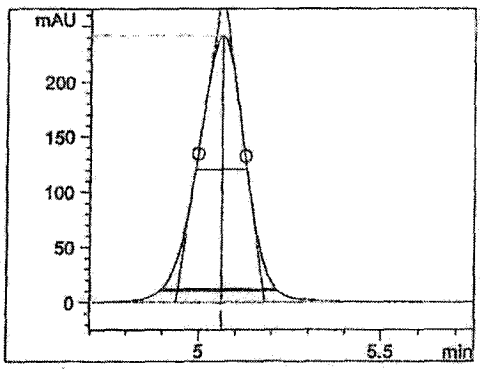
000195

Sample Name: 10-7950A

Compound# 7 : Rebaudioside A
Amount [%] : 97.4375

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.062	k':	-1.000
Height:	241.75	Area:	2251.6
Start:	4.709	End:	5.738
Skew:	0.575	Excess:	4.062
Width at half height:			0.140
5 sigma:			0.320
tangent:			0.244
tailing:			0.311
Symmetry:			1.046
USP Tailing:			0.955
Integration type:			FM
Time increment [msec]:			400.0
Data points:			156



Statistical moments (BB peak detection):

M0:	2235.7
M1:	5.059
M2:	0.004825
M3:	0.000193
M4:	0.000164

Efficiency: Plates per ..

	column	meter
Tangent method	6898	-1
Halfwidth method	7242	-1
5 sigma method	6255	-1
Statistical	5306	-1

Relationship to preceeding peak:

Resolution Tangent method:	2.126	Selectivity: 5 sigma method	1.147 -1.000
Halfwidth method	2.871	Statistical method	3.269

000196

Sample Name: 10-7950A

#	Ret.Time [min]	Amount [%]	Name	Page #
1	2.857	0.0396	Steviolbioside	3
2	3.476	1.5119	Rebaudioside B	4
3	3.977	1.2482	Stevioside	5
4	4.277	0.9018	Rebaudioside C	6
5	4.415	0.7544	Unknown	7
6	5.062	97.4375	Rebaudioside A	8
	Total:	101.8935		

*** End of Report ***

Appendix #8

Reference Standard System Suitability (USP Tailing Factor) (Theoretical Plates)

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-F-08

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: Mariel Esguerra
Date/Time: 10/19/2010 2:35:50 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1FH-0901.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

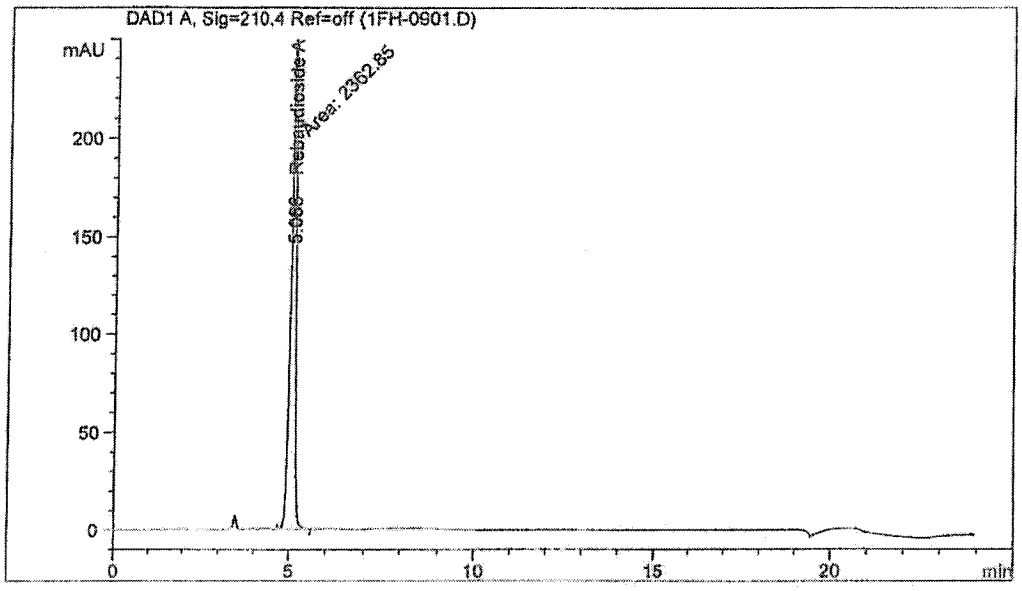
ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

Flow: 1.000 ml/min
Pressure at start: 32 bar
Pressure at end: 35 bar
Left Temp. at start: 35.0°C
Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C
Right Temp. at end: 35.0 °C

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

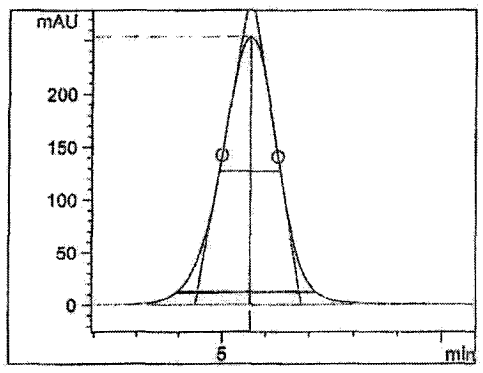


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0898

Peak description [min]:

Signal:	DAD1 A, Sig=210,4 Ref=off		
RetTime:	5.066	k':	-1.000
Height:	253.48	Area:	2362.8
Start:	4.702	End:	5.571
Skew:	0.147	Excess:	1.729
Width at half height:			0.140
5 sigma:			0.324
tangent:			0.245
tailing:			0.313
Symmetry:			1.065
USP Tailing:			0.947
Integration type:			MM
Time increment [msec]:			400.0
Data points:			132



Statistical moments (BB peak detection):

M0:	2355.6
M1:	5.062
M2:	0.004598
M3:	0.000046
M4:	0.0001

Efficiency: Plates per ..

	column	meter
Tangent method	6845	-1
Halfwidth method	7253	-1
5 sigma method	6094	-1
Statistical	5573	-1

Relationship to preceeding peak:

Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.066	2.0898	Rebaudioside A	3
	Total:	2.0898		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647] Copyright © Agilent Technologies

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-F-08

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: Mariel Esguerra
Date/Time: 10/19/2010 5:14:42 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1FH-1301.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

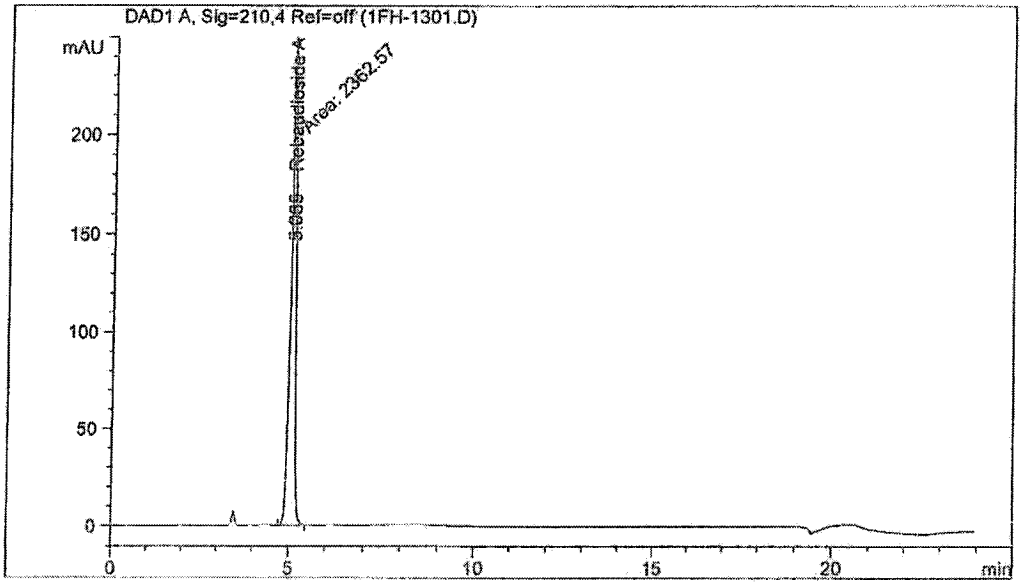
ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

Flow: 1.000 ml/min
Pressure at start: 32 bar
Left Temp. at start: 35.0°C
Right Temp. at start: 35.0°C
Pressure at end: 36 bar
Left Temp. at end: 35.0 °C
Right Temp. at end: 35.0 °C

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

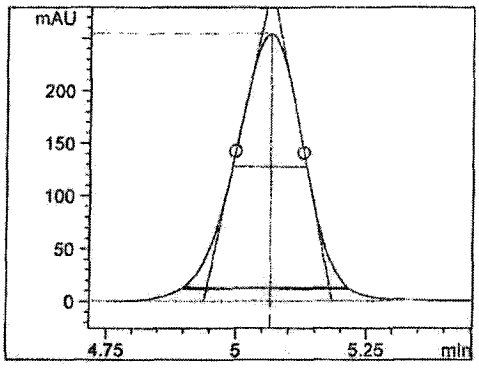


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0895

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.065	k':	-1.000
Height:	254.23	Area:	2362.6
Start:	4.722	End:	5.446
Skew:	0.024	Excess:	1.022
Width at half height:			0.138
5 sigma:			0.322
tangent:			0.244
tailing:			0.315
Symmetry:			1.063
USP Tailing:			0.949
Integration type:			MF
Time increment [msec]:			400.0
Data points:			123



Statistical moments (BB peak detection):

M0:	2352.1
M1:	5.062
M2:	0.00441
M3:	7.15889e-006
M4:	0.000078

Efficiency: Plates per ..

	column	meter
Tangent method	6869	-1
Halfwidth method	7426	-1
5 sigma method	6197	-1
Statistical	5810	-1

Relationship to preceeding peak:

Resolution Tangent method:	-1.000	Selectivity:	-1.000
Halfwidth method	-1.000	5 sigma method	-1.000
		Statistical method	-1.000

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.065	2.0895	Rebaudioside A	7
	Total:	2.0895		

*** End of Report ***

000206

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-F-08

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: Mariel Esguerra
Date/Time: 10/19/2010 7:53:36 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1FH-1701.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

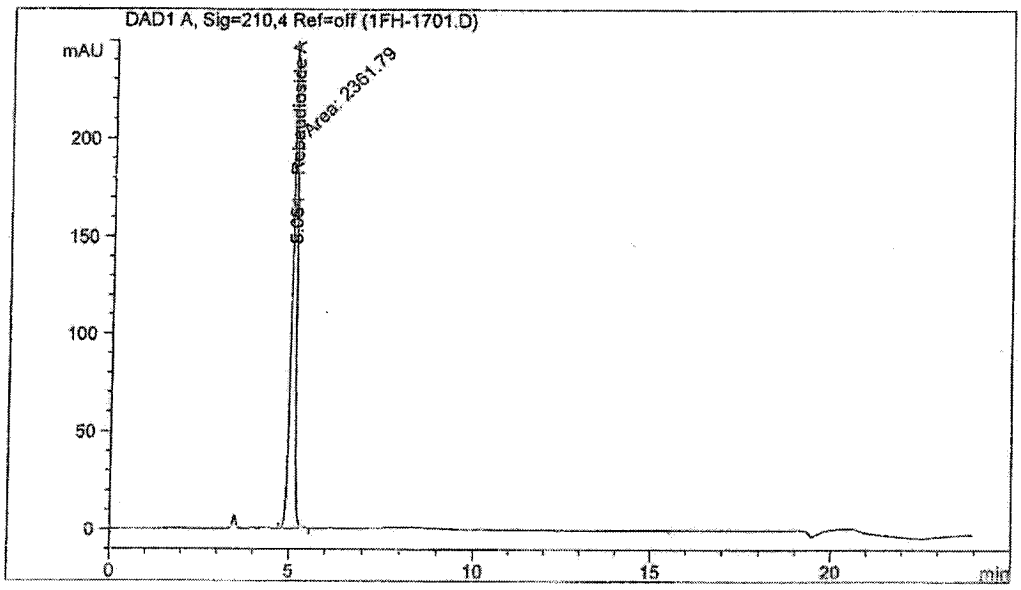
Flow: 1.000 ml/min
Pressure at start: 32 bar
Pressure at end: 35 bar
Left Temp. at start: 35.0°C
Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C
Right Temp. at end: 35.0 °C

000207

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

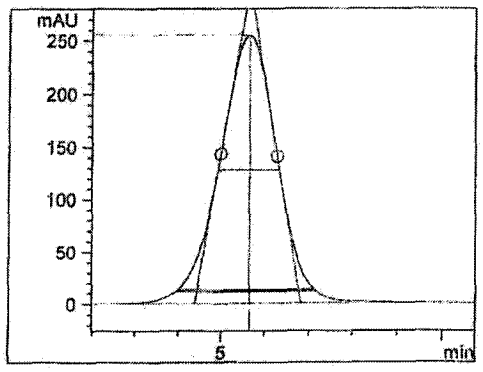


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0888

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.064	k':	-1.000
Height:	254.65	Area:	2361.8
Start:	4.706	End:	5.567
Skew:	0.190	Excess:	1.813
Width at half height:			0.138
5 sigma:			0.322
tangent:			0.244
tailing:			0.313
Symmetry:			1.062
USP Tailing:			0.952
Integration type:			MM
Time increment [msec]:			400.0
Data points:			131



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2361.1			
M1:	5.060	Tangent method	6887	-1
M2:	0.004573	Halfwidth method	7424	-1
M3:	0.000059	5 sigma method	6196	-1
M4:	0.000101	Statistical	5597	-1

Relationship to preceeding peak:

Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.064	2.0888	Rebaudioside A	11
	Total:	2.0888		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-F-08

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: Nicole Leonhardt
Date/Time: 10/19/2010 10:32:32 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1FH-2101.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

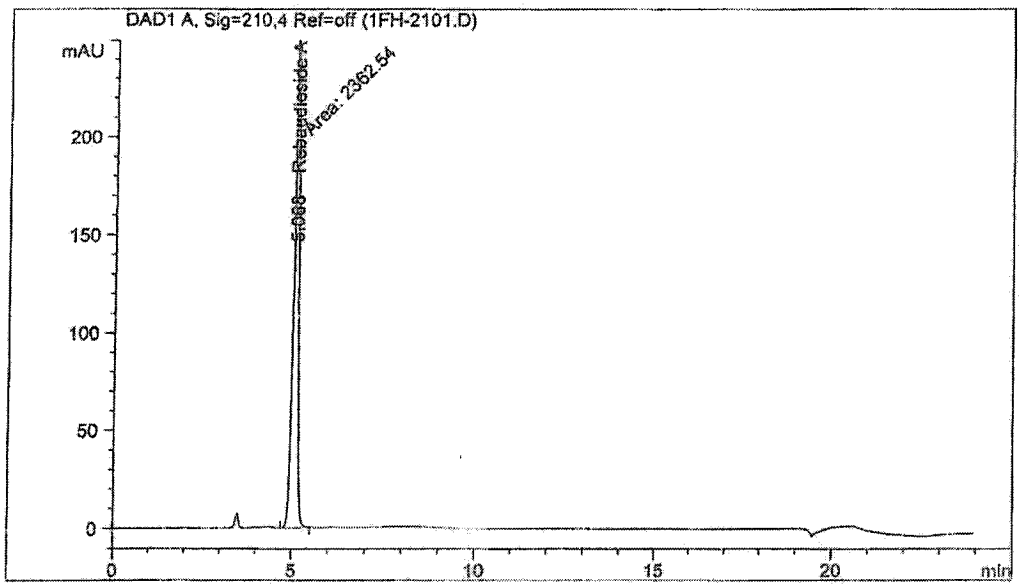
Flow: 1.000 ml/min
Pressure at start: 32 bar
Left Temp. at start: 35.0°C
Right Temp. at start: 35.0°C
Pressure at end: 35 bar
Left Temp. at end: 35.0 °C
Right Temp. at end: 35.0 °C

000211

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

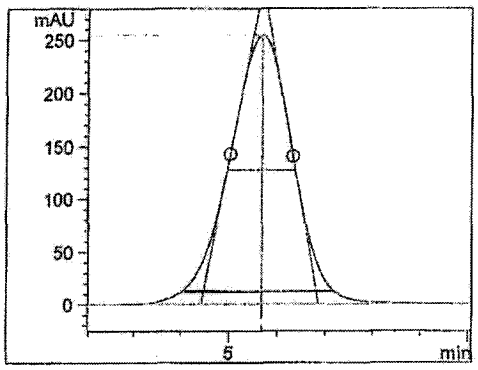


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0895

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.068	k':	-1.000
Height:	254.62	Area:	2362.5
Start:	4.706	End:	5.500
Skew:	0.089	Excess:	1.276
Width at half height:			0.138
5 sigma:			0.322
tangent:			0.244
tailing:			0.313
Symmetry:			1.060
USP Tailing:			0.952
Integration type:			MM
Time increment [msec]:			400.0
Data points:			126



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2355.4			
M1:	5.064	Tangent method	6900	-1
M2:	0.004458	Halfwidth method	7435	-1
M3:	0.000026	5 sigma method	6205	-1
M4:	0.000085	Statistical	5754	-1

Relationship to preceeding peak:

		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

000213

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.068	2.0895	Rebaudioside A	15
	Total:	2.0895		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-F-08

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: Nicole Leonhardt
Date/Time: 10/19/2010 1:11:27 PM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1FH-2501.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

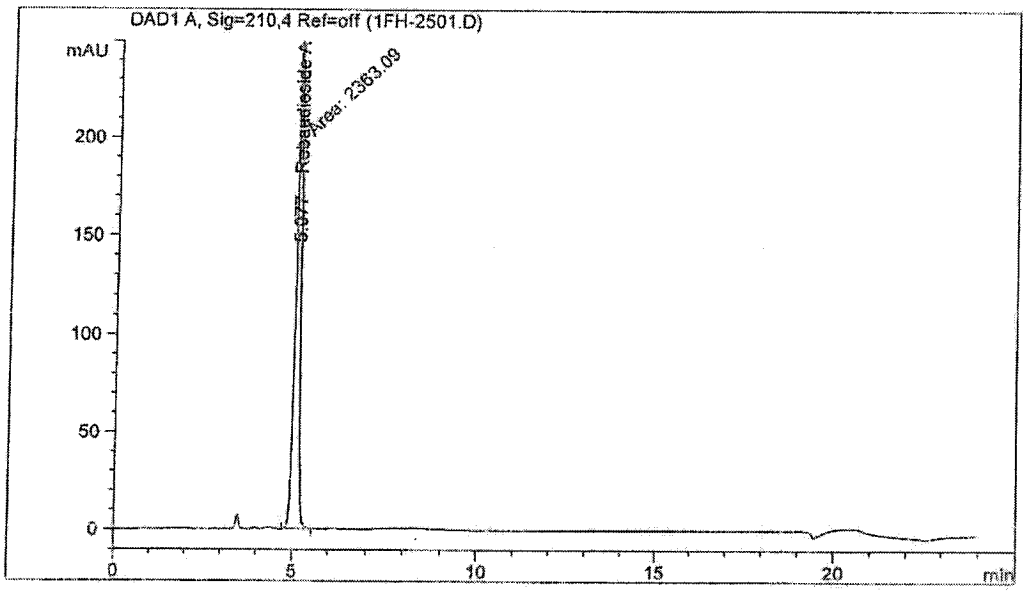
ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

Flow: 1.000 ml/min
Pressure at start: 32 bar
Pressure at end: 35 bar
Left Temp. at start: 35.0°C
Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C
Right Temp. at end: 35.0 °C

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

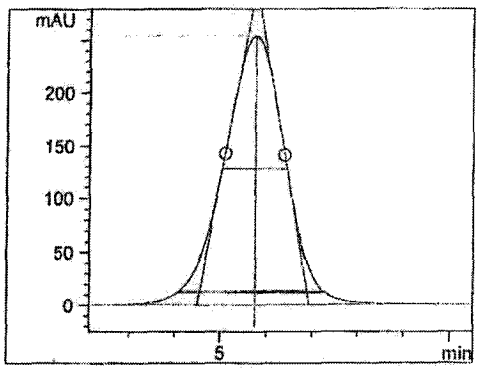


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0900

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.077	k':	-1.000
Height:	254.22	Area:	2363.1
Start:	4.720	End:	5.543
Skew:	0.127	Excess:	1.439
Width at half height:			0.138
5 sigma:			0.322
tangent:			0.245
tailing:			0.313
Symmetry:			1.063
USP Tailing:			0.954
Integration type:			MM
Time increment [msec]:			400.0
Data points:			125



Statistical moments (BB peak detection):

M0:	2362.5		
M1:	5.072	Tangent method	6879
M2:	0.004521	Halfwidth method	7461
M3:	0.000039	5 sigma method	6227
M4:	0.000091	Statistical	5689

Efficiency: Plates per ..

	column	meter
		-1
		-1
		-1
		-1

Relationship to preceeding peak:

Resolution Tangent method:	-1.000	Selectivity:	-1.000
Halfwidth method	-1.000	5 sigma method	-1.000
		Statistical method	-1.000

000217

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.077	2.0900	Rebaudioside A	19
	Total:	2.0900		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: (b) (6) t
Date/Time: 10/19/2010 3:50:21 PM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-2901.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

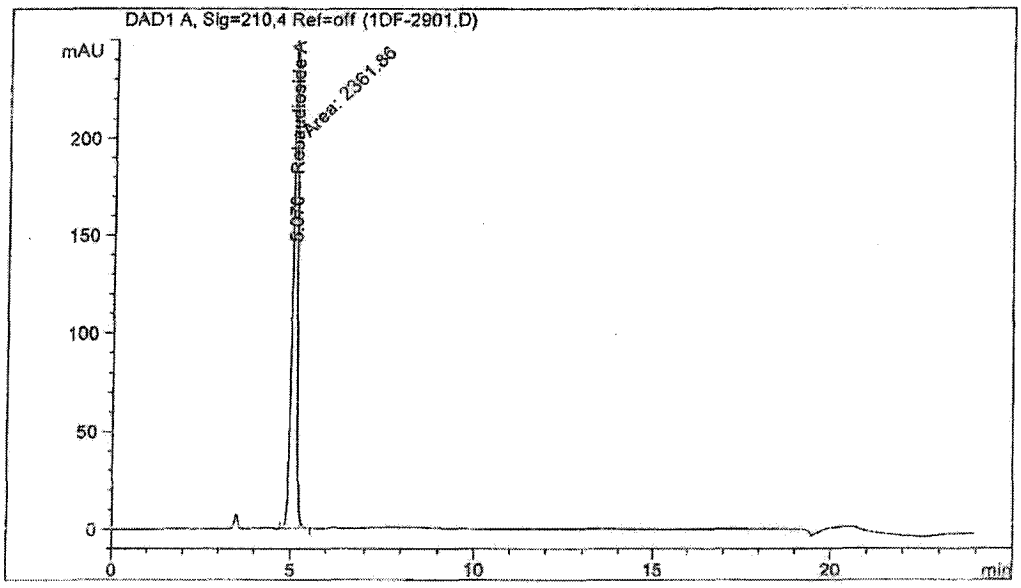
Flow: 1.000 ml/min
Pressure at start: 32 bar
Pressure at end: 35 bar
Left Temp. at start: 35.0°C
Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C
Right Temp. at end: 35.0 °C

000219

Sample Name: Rebaudioside Stk

Solvents:	PMP1 , Solvent ACN/Water (80/20), pH 3.0
	PMP1 , Solvent Milli Q Water
	PMP1 , Solvent
	PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

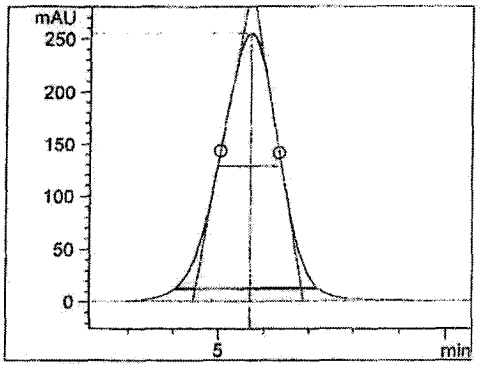


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0889

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.070	k':	-1.000
Height:	254.89	Area:	2361.9
Start:	4.718	End:	5.551
Skew:	0.159	Excess:	1.533
Width at half height:			0.140
5 sigma:			0.322
tangent:			0.244
tailing:			0.312
Symmetry:			1.060
USP Tailing:			0.954
Integration type:			MM
Time increment [msec]:			400.0
Data points:			127



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2361.6			
M1:	5.067	Tangent method	6898	-1
M2:	0.004505	Halfwidth method	7266	-1
M3:	0.000048	5 sigma method	6211	-1
M4:	0.000092	Statistical	5700	-1

Relationship to preceeding peak:

Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.070	2.0889	Rebaudioside A	23
	Total:	2.0889		

*** End of Report ***

000222

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647] Copyright © Agilent Technologies

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name: Rebaudioside Stk Multiplier: 1.00
Injection#: 1 Dilution: 1.00
Injection volume: 5.0 µl

Acquisition information:

Operator: (b) (6) i
Date/Time: 10/19/2010 8:29:16 PM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-3301.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

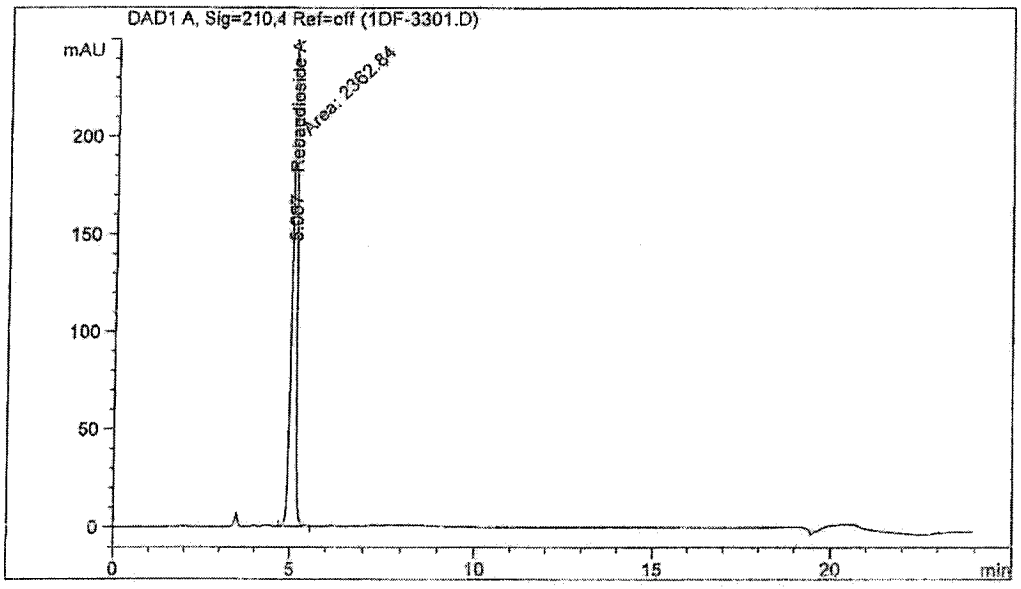
Flow: 1.000 ml/min
Pressure at start: 32 bar Pressure at end: 35 bar
Left Temp. at start: 35.0°C Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C Right Temp. at end: 35.0 °C

000223

Sample Name: Rebaudioside Stk

Solvents:	PMP1 , Solvent ACN/Water (80/20), pH 3.0
	PMP1 , Solvent Milli Q Water
	PMP1 , Solvent
	PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off



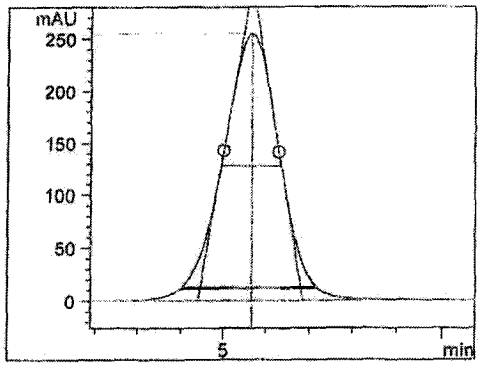
000224

Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0897

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	5.067	k':	-1.000
Height:	254.63	Area:	2362.8
Start:	4.696	End:	5.567
Skew:	0.162	Excess:	1.605
Width at half height:			0.140
5 sigma:			0.320
tangent:			0.244
tailing:			0.311
Symmetry:			1.060
USP Tailing:			0.955
Integration type:			MM
Time increment [msec]:			400.0
Data points:			133



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2358.9			
M1:	5.065	Tangent method	6888	-1
M2:	0.004519	Halfwidth method	7258	-1
M3:	0.000049	5 sigma method	6269	-1
M4:	0.000094	Statistical	5677	-1

Relationship to preceeding peak:

		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.067	2.0897	Rebaudioside A	27
	Total:	2.0897		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name:	Rebaudioside Stk	Multiplier:	1.00
Injection#:	1	Dilution:	1.00

Injection volume: 5.0 µl

Acquisition information:

Operator: (b) (6) i
Date/Time: 10/19/2010 5:08:10 PM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-3701.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

Flow: 1.000 ml/min

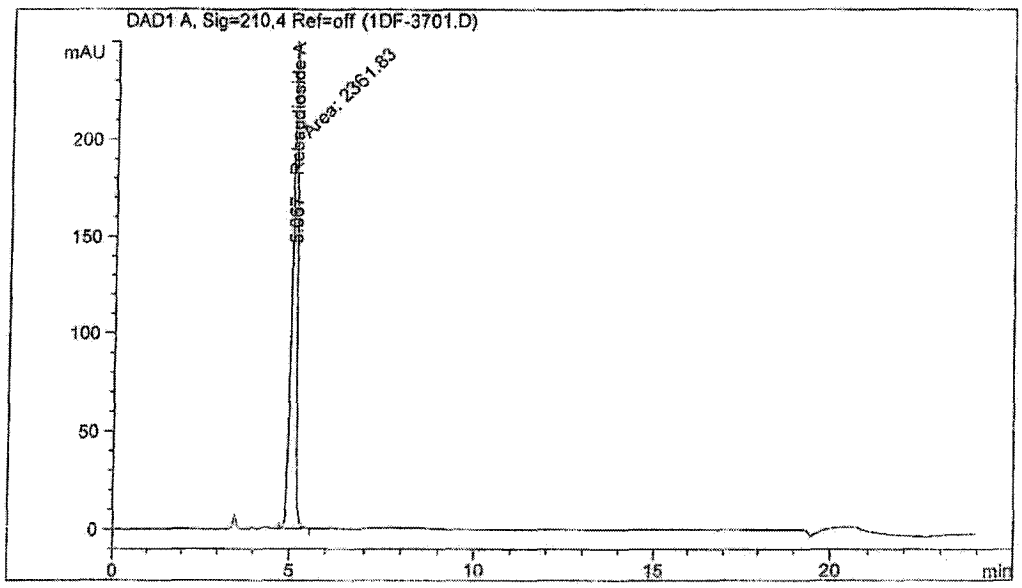
Pressure at start:	32 bar	Pressure at end:	35 bar
Left Temp. at start:	35.0°C	Left Temp. at end:	35.0 °C
Right Temp. at start:	35.0°C	Right Temp. at end:	35.0 °C

000227

Sample Name: Rebaudioside Stk

Solvents:	PMP1 , Solvent ACN/Water (80/20), pH 3.0
	PMP1 , Solvent Milli Q Water
	PMP1 , Solvent
	PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off



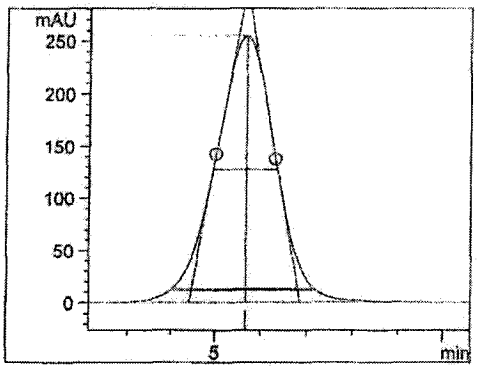
000228

Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0889

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.067	k':	-1.000
Height:	254.89	Area:	2361.8
Start:	4.716	End:	5.555
Skew:	0.160	Excess:	1.556
Width at half height:			0.138
5 sigma:			0.320
tangent:			0.245
tailing:			0.313
Symmetry:			1.060
USP Tailing:			0.952
Integration type:			MM
Time increment [msec]:			400.0
Data points:			128



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2359.5			
M1:	5.067	Tangent method	6849	-1
M2:	0.004502	Halfwidth method	7432	-1
M3:	0.000048	5 sigma method	6267	-1
M4:	0.000092	Statistical	5702	-1

Relationship to preceeding peak:

Resolution Tangent method:	-1.000	Selectivity:	-1.000
Halfwidth method	-1.000	5 sigma method	-1.000
		Statistical method	-1.000

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.067	2.0889	Rebaudioside A	31
	Total:	2.0889		

*** End of Report ***

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: (b) (6) i
Date/Time: 10/19/2010 11:47:07 PM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-4101.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

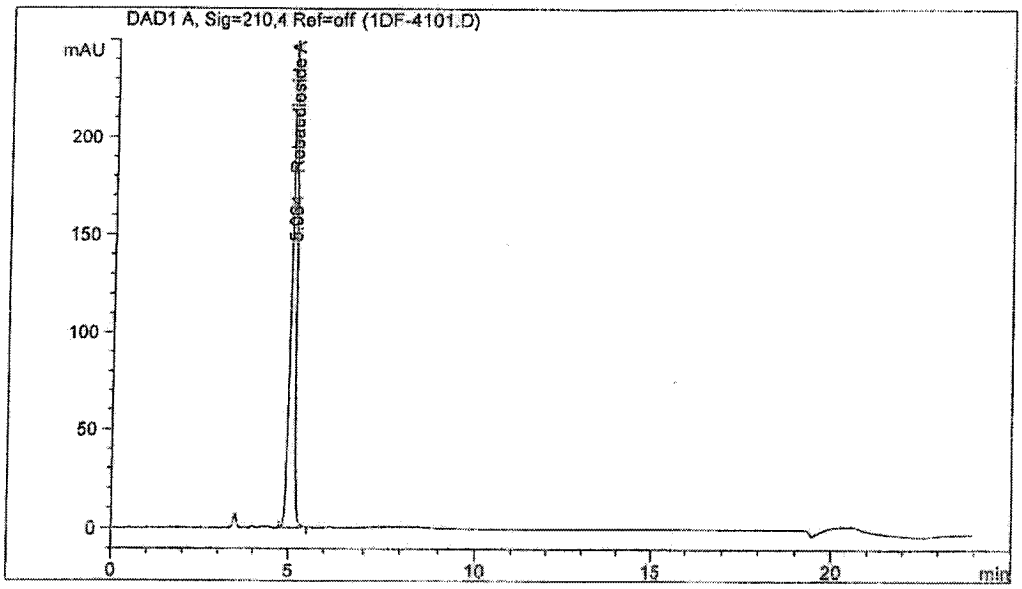
ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

Flow: 1.000 ml/min
Pressure at start: 32 bar
Left Temp. at start: 35.0°C
Right Temp. at start: 35.0°C
Pressure at end: 35 bar
Left Temp. at end: 35.0 °C
Right Temp. at end: 35.0 °C

Sample Name: Rebaudioside Stk

Solvents:	PMP1 , Solvent ACN/Water (80/20), pH 3.0
	PMP1 , Solvent Milli Q Water
	PMP1 , Solvent
	PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off



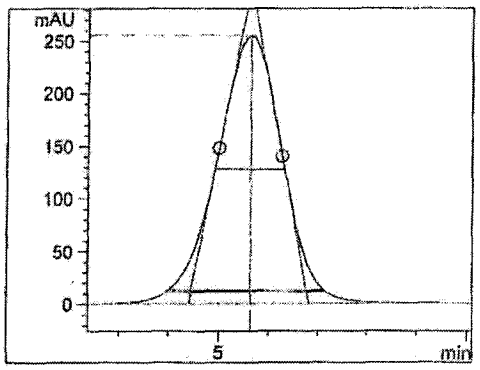
000232

Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0897

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime:	5.064	k':	-1.000
Height:	255.22	Area:	2362.8
Start:	4.743	End:	5.503
Skew:	0.116	Excess:	1.249
Width at half height:			0.138
5 sigma:			0.320
tangent:			0.243
tailing:			0.313
Symmetry:			1.057
USP Tailing:			0.956
Integration type:			BB
Time increment [msec]:			400.0
Data points:			116



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2360.7			
M1:	5.061	Tangent method	6939	-1
M2:	0.004429	Halfwidth method	7423	-1
M3:	0.000034	5 sigma method	6260	-1
M4:	0.000083	Statistical	5783	-1

Relationship to preceeding peak:

		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

000233

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.064	2.0897	Rebaudioside A	35
	Total:	2.0897		

*** End of Report ***

000234

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name: Rebaudioside Stk Multiplier: 1.00
Injection#: 1 Dilution: 1.00
Injection volume: 5.0 µl

Acquisition information:

Operator: (b) (6) i
Date/Time: 10/20/2010 2:26:04 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-4501.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

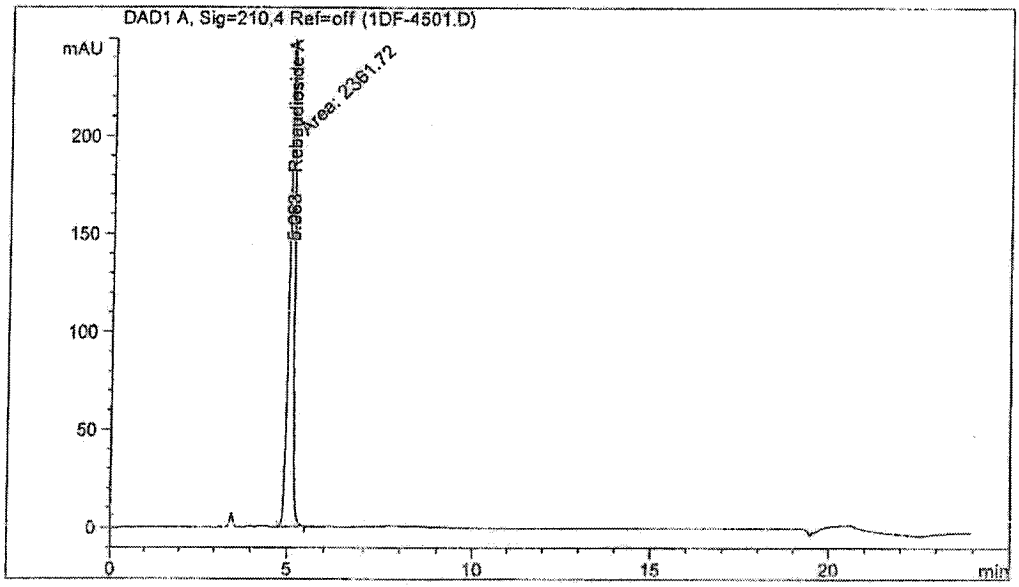
Flow: 1.000 ml/min
Pressure at start: 32 bar Pressure at end: 35 bar
Left Temp. at start: 35.0°C Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C Right Temp. at end: 35.0 °C

000235

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

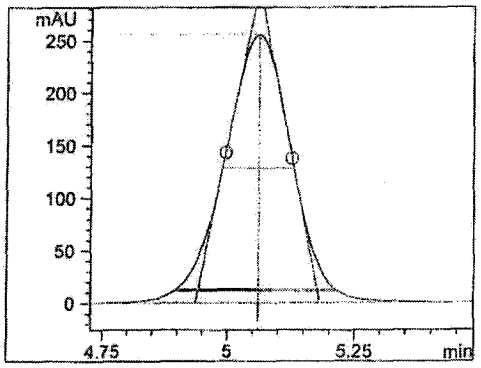


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0888

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.063	k':	-1.000
Height:	255.60	Area:	2361.7
Start:	4.733	End:	5.476
Skew:	0.095	Excess:	1.166
Width at half height:			0.138
5 sigma:			0.320
tangent:			0.245
tailing:			0.312
Symmetry:			1.060
USP Tailing:			0.957
Integration type:			MM
Time increment [msec]:			400.0
Data points:			120



Statistical moments (BB peak detection):

			Efficiency: Plates per .. column	meter
M0:	2361.6			
M1:	5.058	Tangent method	6852	-1
M2:	0.004405	Halfwidth method	7420	-1
M3:	0.000028	5 sigma method	6257	-1
M4:	0.000081	Statistical	5807	-1

Relationship to preceeding peak:

Selectivity:	-1.000		
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

000237

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.063	2.0888	Rebaudioside A	39
	Total:	2.0888		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647]

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name: Rebaudioside Stk
Injection#: 1
Injection volume: 5.0 µl
Multiplier: 1.00
Dilution: 1.00

Acquisition information:

Operator: (b) (6) i
Date/Time: 10/28/2010 5:04:59 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-4901.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

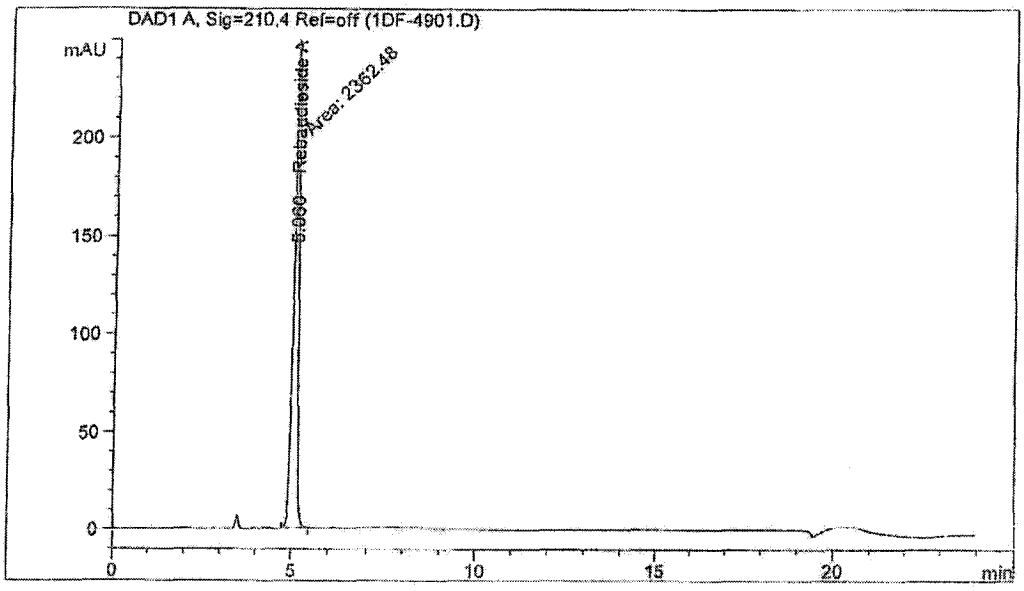
Flow: 1.000 ml/min
Pressure at start: 32 bar
Left Temp. at start: 35.0°C
Right Temp. at start: 35.0°C
Pressure at end: 35 bar
Left Temp. at end: 35.0 °C
Right Temp. at end: 35.0 °C

000239

Sample Name: Rebaudioside Stk

Solvents: PMP1 , Solvent ACN/Water (80/20), pH 3.0
PMP1 , Solvent Milli Q Water
PMP1 , Solvent
PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

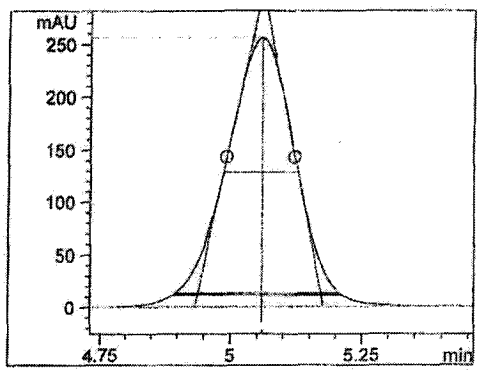


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0894

Peak description [min]:

Signal: DAD1 A, Sig=210,4 Ref=off			
RetTime: 5.060	k':	-1.000	
Height: 255.93	Area:	2362.5	
Start: 4.737	End:	5.454	
Skew: 0.077	Excess:	1.080	
Width at half height:		0.140	
5 sigma:		0.320	
tangent:		0.244	
tailing:		0.312	
Symmetry:		1.059	
USP Tailing:		0.954	
Integration type:		MM	
Time increment [msec]:		400.0	
Data points:		120	



Statistical moments (BB peak detection):

			Efficiency: Plates per ..	
			column	meter
M0:	2362.1			
M1:	5.059	Tangent method	6905	-1
M2:	0.004376	Halfwidth method	7236	-1
M3:	0.000022	5 sigma method	6250	-1
M4:	0.000078	Statistical	5848	-1

Relationship to preceeding peak:

		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

000241

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.060	2.0894	Rebaudioside A	43
	Total:	2.0894		

*** End of Report ***

Sample Name: Rebaudioside Stk

Extended Performance Report

Instrument: HPLC 10

Module	Type	Firmware rev.	Serial number
1100/1200 Quaternary Pump	G1311A	A.06.10 [005]	DE43632199
1100 Wellplate Autosampler	G1367A	A.06.12 [003]	DE50404329
1100/1200 Column Thermostat	G1316A	A.06.10 [004]	DE43646355
1100/1200 Diode Array Detector	G1315B	A.06.10 [004]	DE43625486
1200 Sample Thermostat	G1330B	n/a	DE13211310

Software Revision: Rev. B.04.01 SP1 [647] Copyright © Agilent Technologies

Analysis method:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Name: STEVIA.M

Sample information for Location: P1-D-06

Sample Name: Rebaudioside Stk Multiplier: 1.00
Injection#: 1 Dilution: 1.00
Injection volume: 5.0 µl

Acquisition information:

Operator: (b) (6) i
Date/Time: 10/20/2010 9:03:26 AM
Data file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: 1DF-5501.D
Method file:
Path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
Name: STEVIA.M

ECM Information: Server: http://us05sqlc Operator: Mariel Esguerra
Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version: 1

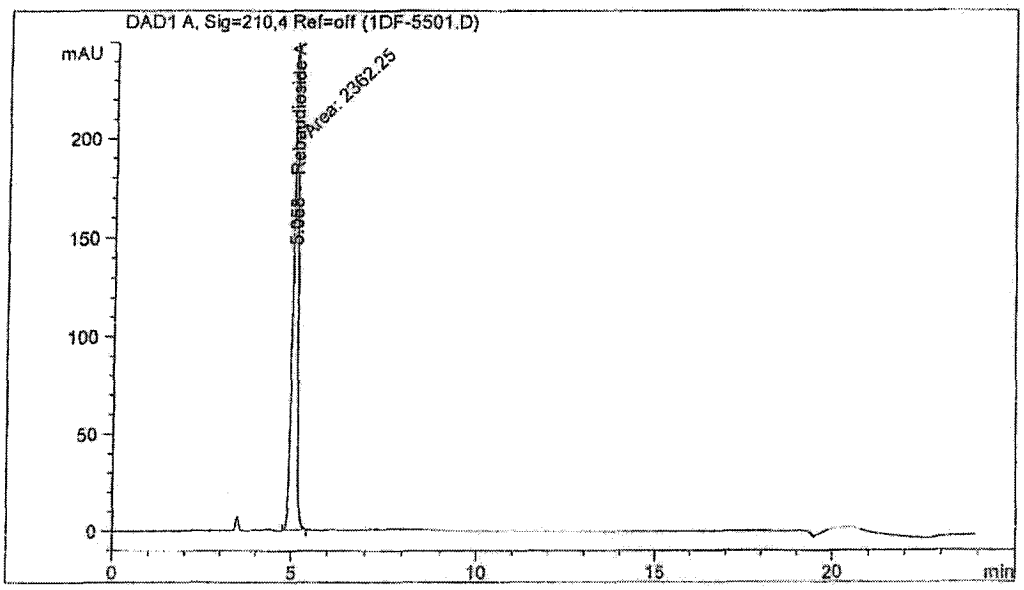
Flow: 1.000 ml/min
Pressure at start: 32 bar Pressure at end: 35 bar
Left Temp. at start: 35.0°C Left Temp. at end: 35.0 °C
Right Temp. at start: 35.0°C Right Temp. at end: 35.0 °C

000243

Sample Name: Rebaudioside Stk

Solvents:	PMP1 , Solvent ACN/Water (80/20), pH 3.0
	PMP1 , Solvent Milli Q Water
	PMP1 , Solvent
	PMP1 , Solvent

Signal description: DAD1 A, Sig=210,4 Ref=off

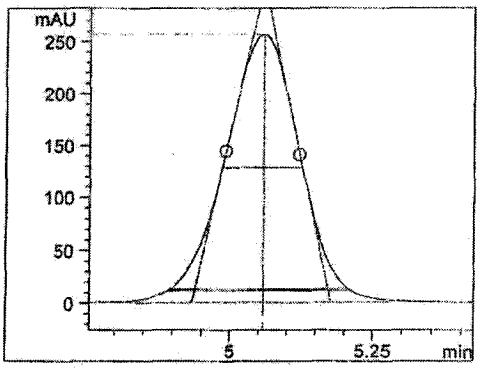


Sample Name: Rebaudioside Stk

Compound# 7 : Rebaudioside A
Amount [mg/mL]: 2.0892

Peak description [min]:
Signal: DAD1 A, Sig=210,4 Ref=off

RetTime:	5.058	k':	-1.000
Height:	256.51	Area:	2362.2
Start:	4.759	End:	5.415
Skew:	0.041	Excess:	0.826
Width at half height:			0.140
5 sigma:			0.318
tangent:			0.243
tailing:			0.310
Symmetry:			1.060
USP Tailing:			0.960
Integration type:			MM
Time increment [msec]:			400.0
Data points:			118



Statistical moments (BB peak detection):

			Efficiency: Plates per ..	
			column	meter
M0:	2362.7			
M1:	5.053	Tangent method	6917	-1
M2:	0.004279	Halfwidth method	7232	-1
M3:	0.000011	5 sigma method	6312	-1
M4:	0.00007	Statistical	5967	-1

Relationship to preceeding peak:

		Selectivity:	-1.000
Resolution Tangent method:	-1.000	5 sigma method	-1.000
Halfwidth method	-1.000	Statistical method	-1.000

000245

Sample Name: Rebaudioside Stk

#	Ret.Time [min]	Amount [mg/mL]	Name	Page #
1	5.058	2.0892	Rebaudioside A	47
	Total:	2.0892		

*** End of Report ***

000246

Statistic Report

Bracketing Standards only.

mac 08Dec10

Sequence table: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA-7941.S
Data directory path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Ver: 1
Operator: Mariel Esguerra

Method file name: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M

Run #	Location	Inj #	Inj. Date/Time	File Name	Sample Name
1	P1-F-08	1	10/19/2010 2:35:50 AM	1FH-0901.D	Rebaudioside Stk
2	P1-F-08	1	10/19/2010 5:14:42 AM	1FH-1301.D	Rebaudioside Stk
3	P1-F-08	1	10/19/2010 7:53:36 AM	1FH-1701.D	Rebaudioside Stk
4	P1-F-08	1	10/19/2010 10:32:32 AM	1FH-2101.D	Rebaudioside Stk
5	P1-F-08	1	10/19/2010 1:11:27 PM	1FH-2501.D	Rebaudioside Stk
6	P1-D-06	1	10/19/2010 3:50:21 PM	1DF-2901.D	Rebaudioside Stk
7	P1-D-06	1	10/19/2010 6:29:16 PM	1DF-3301.D	Rebaudioside Stk
8	P1-D-06	1	10/19/2010 9:08:10 PM	1DF-3701.D	Rebaudioside Stk
9	P1-D-06	1	10/19/2010 11:47:07 PM	1DF-4101.D	Rebaudioside Stk
10	P1-D-06	1	10/20/2010 2:26:04 AM	1DF-4501.D	Rebaudioside Stk
11	P1-D-06	1	10/20/2010 5:04:59 AM	1DF-4901.D	Rebaudioside Stk
12	P1-D-06	1	10/20/2010 9:03:26 AM	1DF-5501.D	Rebaudioside Stk

Statistic results for compound Steviolbioside not available.

Statistic results for compound Dulcoside A not available.

Statistic results for compound Rebaudioside B not available.

Statistic results for compound Stevioside not available.

Statistic results for compound Rebaudioside C not available.

Statistic results for compound Unknown not available.

Compound: Rebaudioside A (Signal: DAD1 A, Sig=210,4 Ref=off)

Run #	Type	RetTime [min]	Amount [mg/mL]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
1	MM	5.066	2.08976	2362.84985	253.48116	0.1400	1.07
2	MF	5.065	2.08950	2362.56714	254.23129	0.1383	1.06
3	MM	5.064	2.08884	2361.79150	254.65474	0.1383	1.06
4	MM	5.068	2.08950	2362.53833	254.62416	0.1383	1.06
5	MM	5.077	2.08995	2363.08594	254.22134	0.1383	1.06
6	MM	5.070	2.08889	2361.86304	254.88712	0.1400	1.06
7	MM	5.067	2.08974	2362.84155	254.63269	0.1400	1.06
8	MM	5.067	2.08887	2361.83228	254.88556	0.1383	1.06
9	BB	5.064	2.08969	2362.77563	255.22141	0.1383	1.06
10	MM	5.063	2.08878	2361.71802	255.60187	0.1383	1.06
11	MM	5.060	2.08944	2362.47607	255.92769	0.1400	1.06
12	MM	5.058	2.08924	2362.24634	256.51361	0.1400	1.06

000247

	RetTime [min]	Amount [mg/mL]	Area [mAU*s]	Height [mAU]	Width [min]	Symm.
Mean:	5.066	2.08935	2362.38214	254.90689	0.1390	1.06
S.D.:	4.80e-3	4.14578e-4	4.79356e-1	8.19322e-1	8.58e-4	2e-3
RSD :	0.095	1.98424e-2	2.02912e-2	3.21420e-1	0.6173	0.21
95% CI:	3.05e-3	2.63410e-4	3.04568e-1	5.20572e-1	5.45e-4	1e-3

S t a t i s t i c R e p o r t

Sequence table: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA-7941.S
Data directory path: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\
ECM Host: http://us05sqlc
ECM Path: Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Ver: 1
Operator: Mariel Esguerra

No (or not enough) sample runs with cal. compounds available!

GR 

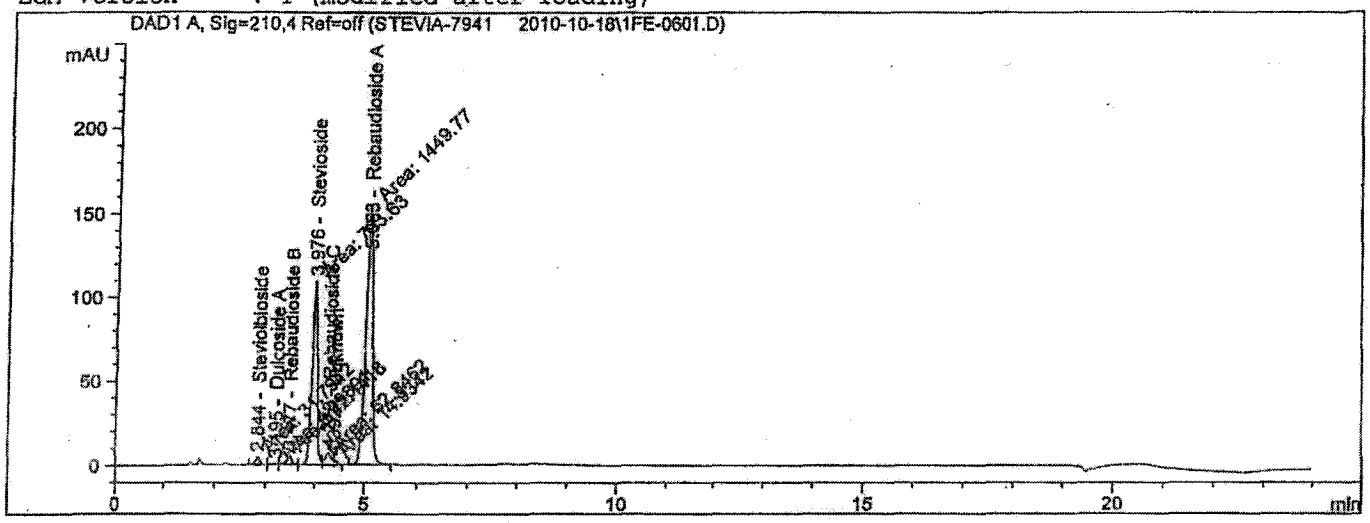
Appendix #9

Accuracy

000250

Acq. Operator : Mariel Esguerra Seq. Line : 6
Acq. Instrument : HPLC 10 Location : P1-F-05
Injection Date : 10/19/2010 12:36:40 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 12:37:41 PM by Mariel Esguerra
(modified after loading)
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (modified after loading)



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:34:47 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.00350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.844	MF	31.79421	5.83724e-4	0.926331		Steviolbioside
3.195	MF	4.09891	7.25233e-4	0.148374		Dulcoside A
3.477	MF	42.14180	7.34077e-4	1.544064		Rebaudioside B
3.976	MF	793.63043	7.34077e-4	29.078405		Stevioside
4.279	MF	62.84620	8.66741e-4	2.718811		Rebaudioside C
4.392	MF	14.93418	8.84430e-4	0.659258		Unknown
5.063	MF	1449.76501	8.84433e-4	63.999018		Rebaudioside A

Totals : 99.074261

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

==========

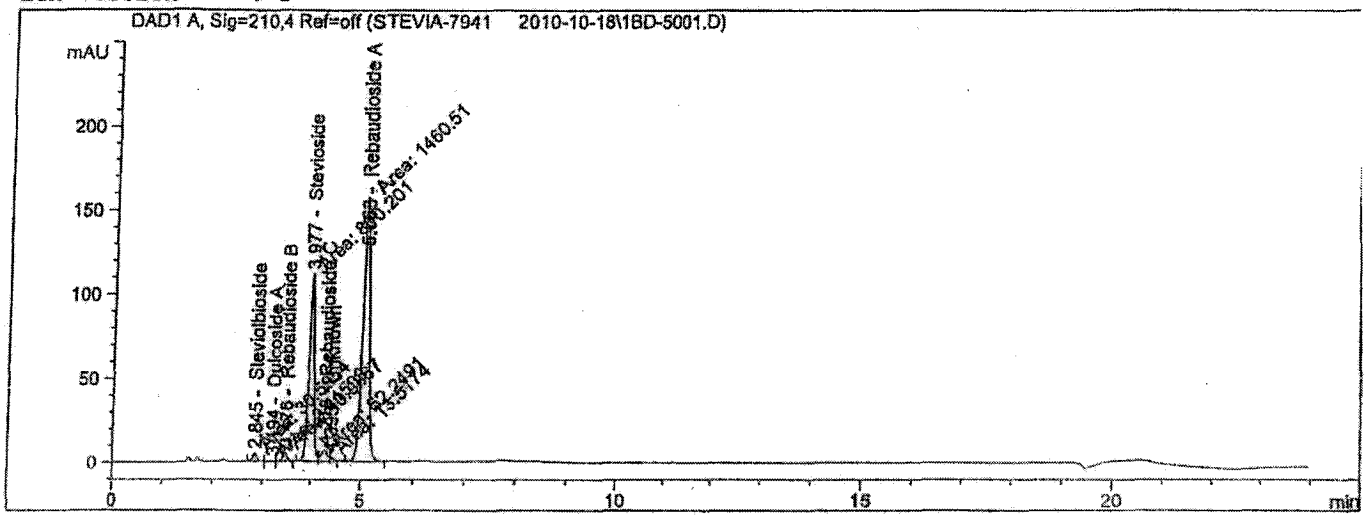
*** End of Report ***

Acq. Operator : (b) (6)
Acq. Instrument : HPLC 10
Injection Date : 10/20/2010 5:44:43 AM

Seq. Line : 50
Location : P1-B-04
Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 12:37:56 PM by Mariel Esguerra
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 12:34:47 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.00350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.845	MF	30.59544	5.83724e-4	0.891405		Steviolbioside
3.194	MF	3.41503	7.25233e-4	0.123618		Dulcoside A
3.476	MF	40.93667	7.34077e-4	1.499909		Rebaudioside B
3.977	FM	800.20105	7.34077e-4	29.319151		Stevioside
4.278	MF	62.24906	8.66741e-4	2.692978		Rebaudioside C
4.395	MF	13.51745	8.84430e-4	0.596718		Unknown
5.063	MF	1460.51331	8.84433e-4	64.473495		Rebaudioside A
Totals :				99.597273		

1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)

==========

*** End of Report ***

Results follow:

	Day 1	Day 2	Day 3
Retention time (Rt) Range (minutes)	15.786-16.465	15.673-15.720	15.348-15.579
Rt % RSD	1.664	0.131	0.554
Rebaudioside A Peak Area RSD	0.300	0.161	0.898
Number of Data Points	8	7	7

Rebaudioside A Retention Time Range meets the criteria of deviation of less than 1 minute.
Rebaudioside A Retention time % RSD = PASS.
Rebaudioside A Peak Area RSD, all PASS with results of less than 1.5 percent as per the method.

3. An Extended Performance report was generated using Agilent Chem Station software to include resolution, tailing and theoretical plate counts, comparing stevioside to rebaudioside A (Reb A). Results were determined at the beginning and end of the Day 1 runs. Results are as follows;

Beginning of run;

USP Resolution Stevioside/ Reb A = 1.156
USP Tailing Stevioside = 1.048
USP Tailing Reb A = 1.051
USP Plate Count Tangent Method, 11843/11267

End of run;

USP Resolution Stevioside/ Reb A = 1.156
USP Tailing Stevioside = 1.055
USP Tailing Reb A = 1.054
USP Plate Count Tangent Method, 12175/11784

4. The retention time and identity for Rebaudioside A was confirmed using the ChromaDex rebaudioside A standard. Chromatograms are located in the accuracy portion of the package.

F. Accuracy:

Accuracy was determined by applying the analytical procedure to an analyte of known purity. For this purpose a Chromadex Rebaudioside A standard of known purity was used. Per ICH recommendations, a minimum of 9 determinations each was performed on three concentration levels covering the range of the method (e.g., 3 concentrations/3 replicates).

1. Accuracy stock standards:

The ChromaDex rebaudioside A standard was diluted separately on three days Stock concentrations used on each day are listed here:

Stock	Concentration (mg/ml)
Day 1	1.955626
Day 2	1.945567
Day 3	1.939763

The accuracy stock standards were diluted 1:2 and 1:4 to complete the mid and low level standards. Concentrations are listed in the associated table below with the results for the accuracy tests:

Accuracy Continued:

2. Standard concentrations with accuracy results:

ChromaDex Lot Number	Concentration (mg/ml)	Stevioside Result, Percent (%w/w)	Day Tested
18226-584	1.956	101	Day1
18226-584	1.946	97.9	Day2
18226-584	1.94	101.1	Day3
18226-584	0.9778	101.3	Day1
18226-584	0.9728	98.4	Day2
18226-584	0.9699	101.9	Day3
18226-584	0.4889	100.4	Day1
18226-584	0.4864	99.2	Day2
18226-584	0.4849	101.9	Day3

RSD between levels = 1.49

3. Accuracy Acceptance criteria:
   a. Recoveries must be 98 -102%
   b. RSD between levels must be ≤ 5%.

All results meet the criteria for % recovery. The average percent recovery over all 9 determinations is 100.3%. The RSD calculated on all nine data points is 1.49%.

G. Repeatability:

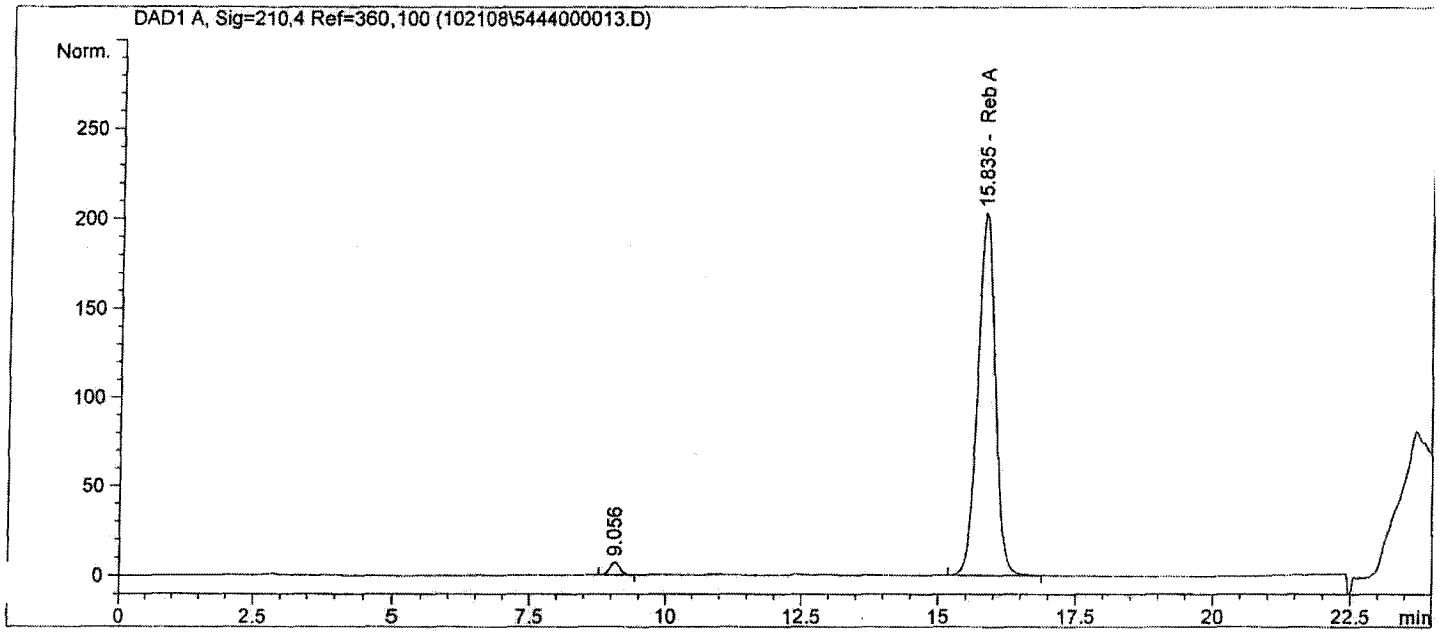
1. For the sample, perform 5 sample preparations. Repeat over 2 separate days, for a total of 10 per matrix. Results follow:

Lot # 33308092926 Eurofins Sample # 08-5444	Amount (mg)	Final Volume	Concentration (mg/mL)	Reb A Result (% w/w) as is
Day1-1	40.22	40	1.0055	93.9
Day1-2	42.04	40	1.0510	94.2
Day1-3	40.94	40	1.0235	93.5
Day1-4	41.37	40	1.03425	93.4

Sample Name: Accuracy/tR

Accuracy Dog 1

Injection Date	: 10/21/2008 9:30:10 PM	Seq. Line	: 10
Sample Name	: Accuracy/tR	Location	: P1-F-09
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/21/2008 1:30:06 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/22/2008 9:26:14 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/22/2008 9:21:18 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.95600 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.864		-	-	-		Stevioside
15.835	BB	4547.33887	4.34397e-4	100.989170		Reb A
Totals :				100.989170		

1 Warnings or Errors :

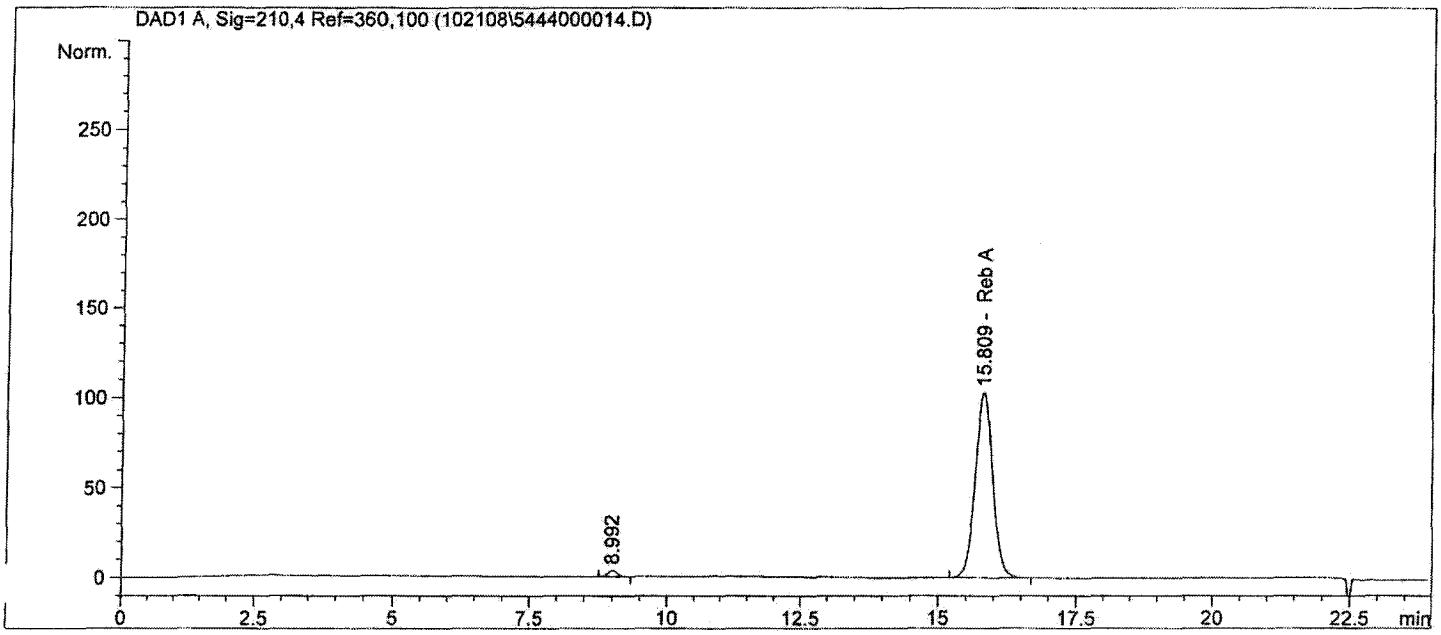
Warning : Calibrated compound(s) not found

*** End of Report ***

000257

Sample Name: Accuracy/tR 1:2

Injection Date	: 10/21/2008 10:05:02 PM	Seq. Line	: 11
Sample Name	: Accuracy/tR 1:2	Location	: P1-E-01
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/21/2008 1:30:06 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/22/2008 9:31:16 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/22/2008 9:21:18 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 9.77800e-1 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.864		-	-	-		Stevioside
15.809	BB	2279.28345	4.34397e-4	101.259234		Reb A
Totals :				101.259234		

1 Warnings or Errors :

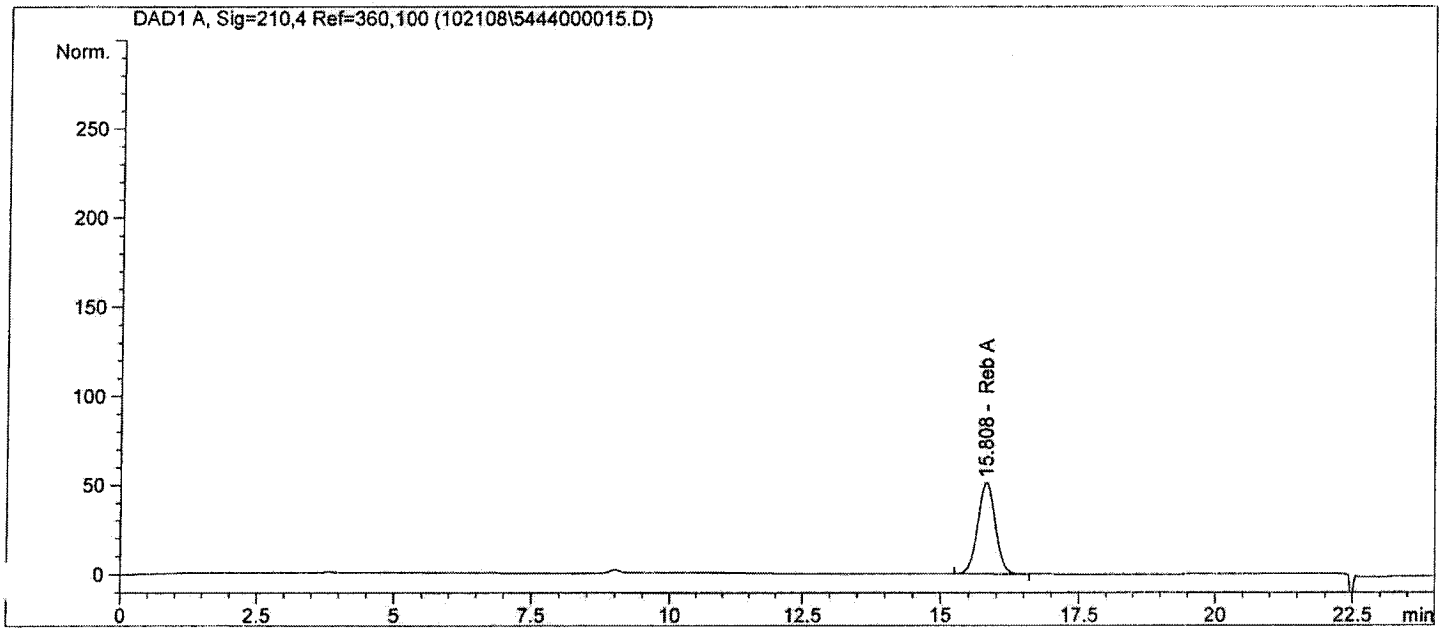
Warning : Calibrated compound(s) not found

*** End of Report ***

000258

Sample Name: Accuracy/tR 1:4

Injection Date	: 10/21/2008 10:39:56 PM	Seq. Line	: 12
Sample Name	: Accuracy/tR 1:4	Location	: P1-E-02
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/21/2008 1:30:06 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/22/2008 9:40:43 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/22/2008 9:21:18 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 4.88900e-1 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.864		-	-	-		Stevioside
15.808	BB	1129.95129	4.34397e-4	100.398222		Reb A
Totals :				100.398222		

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

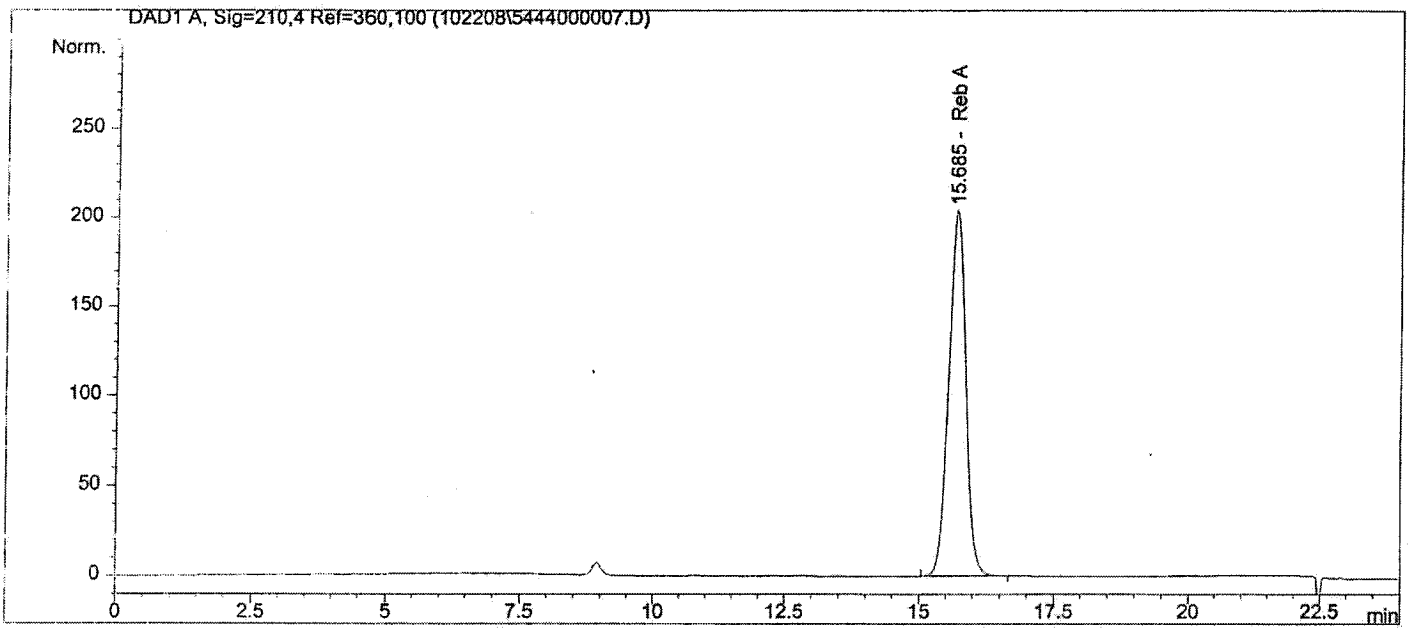
*** End of Report ***

000259

Data File F:\HPLC10\DATA\102208\5444000007.D
Sample Name: Accuracy

Accuracy day 2

Injection Date	: 10/22/2008 8:51:50 PM	Seq. Line	: 4
Sample Name	: Accuracy	Location	: P1-F-03
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/22/2008 3:21:08 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/23/2008 10:17:04 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/23/2008 10:17:38 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.94600 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.777		-	-	-		Stevioside
15.685	BB	4505.11914	4.22975e-4	97.921504		Reb A
Totals :				97.921504		

1 Warnings or Errors :

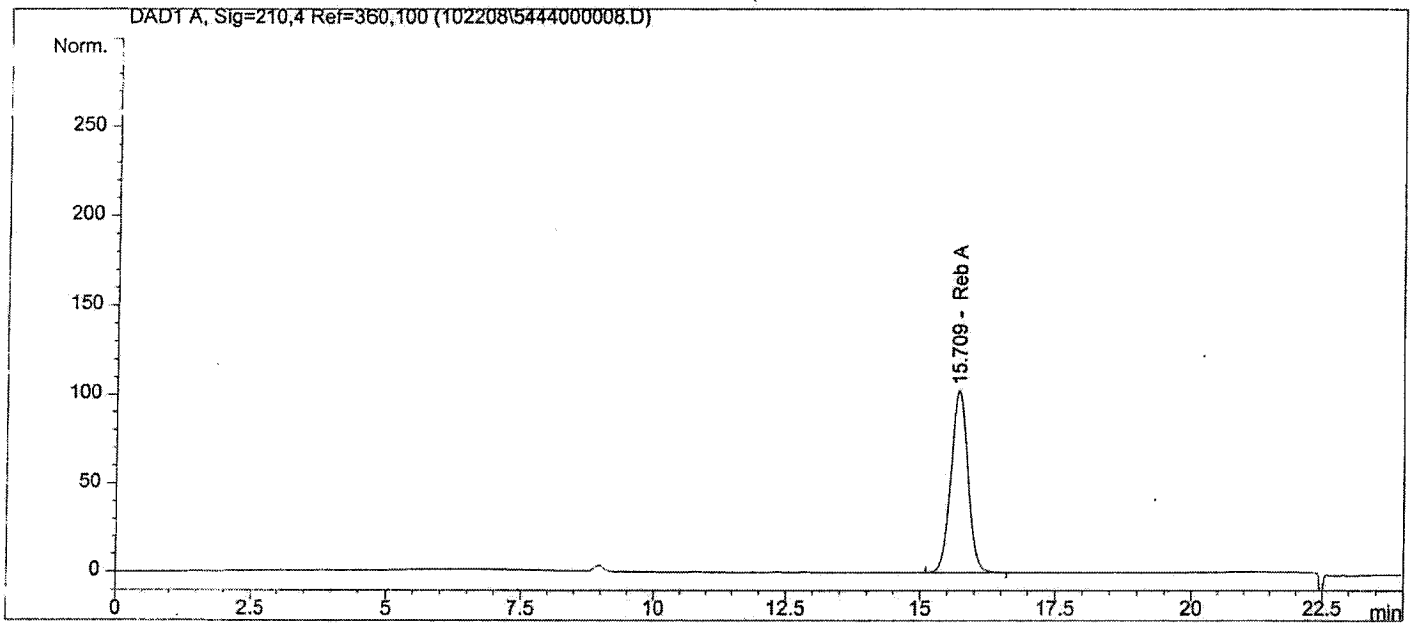
Warning : Calibrated compound(s) not found

*** End of Report ***

000260

Sample Name: Accuracy 1:2

Injection Date	: 10/22/2008 9:26:49 PM	Seq. Line	: 5
Sample Name	: Accuracy 1:2	Location	: P1-F-04
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/22/2008 3:21:08 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/23/2008 10:18:16 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/23/2008 10:17:38 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 9.72783e-1 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.777		-	-	-		Stevioside
15.709	BB	2261.92627	4.22975e-4	98.350620		Reb A
Totals :				98.350620		

1 Warnings or Errors :

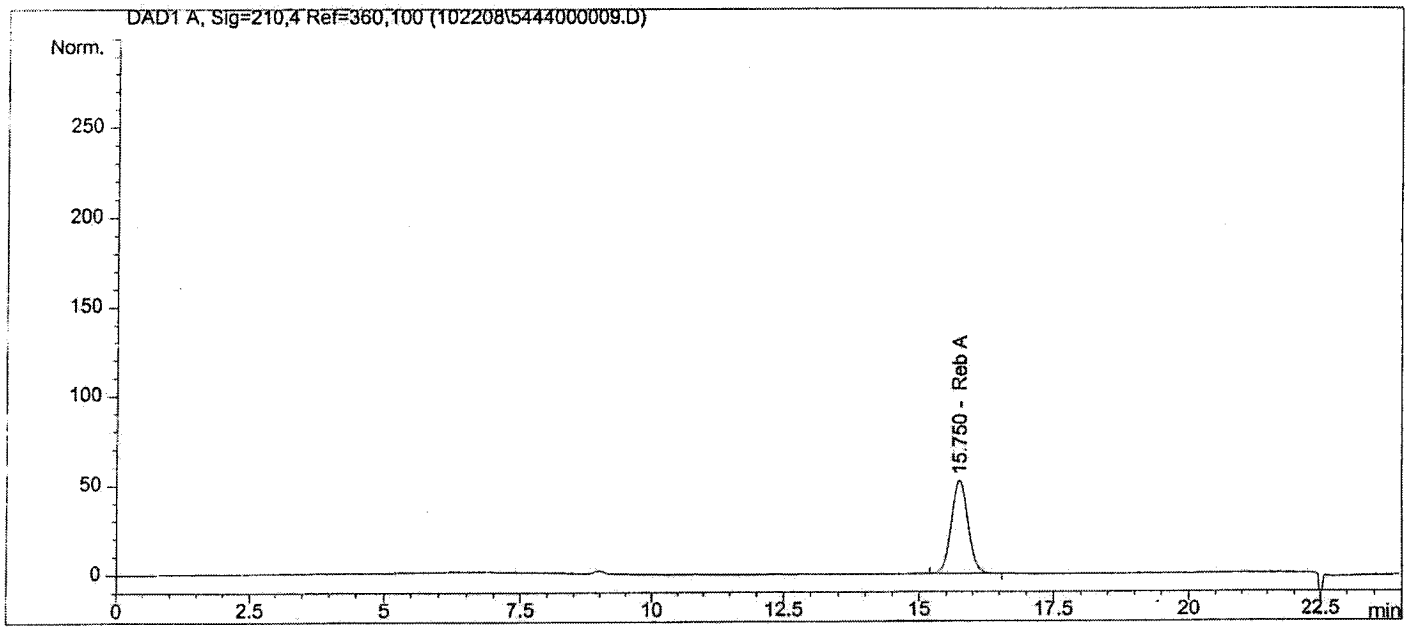
Warning : Calibrated compound(s) not found

*** End of Report ***

000261

Data File F:\HPLC10\DATA\102208\5444000009.D
Sample Name: Accuracy 1:4

Injection Date	: 10/22/2008 10:01:42 PM	Seq. Line	: 6
Sample Name	: Accuracy 1:4	Location	: P1-F-05
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/22/2008 3:21:08 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/23/2008 10:18:43 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/23/2008 10:17:38 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 4.86392e-1 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.777		-	-	-		Stevioside
15.750	BB	1140.35913	4.22975e-4	99.167611		Reb A
Totals :				99.167611		

1 Warnings or Errors :

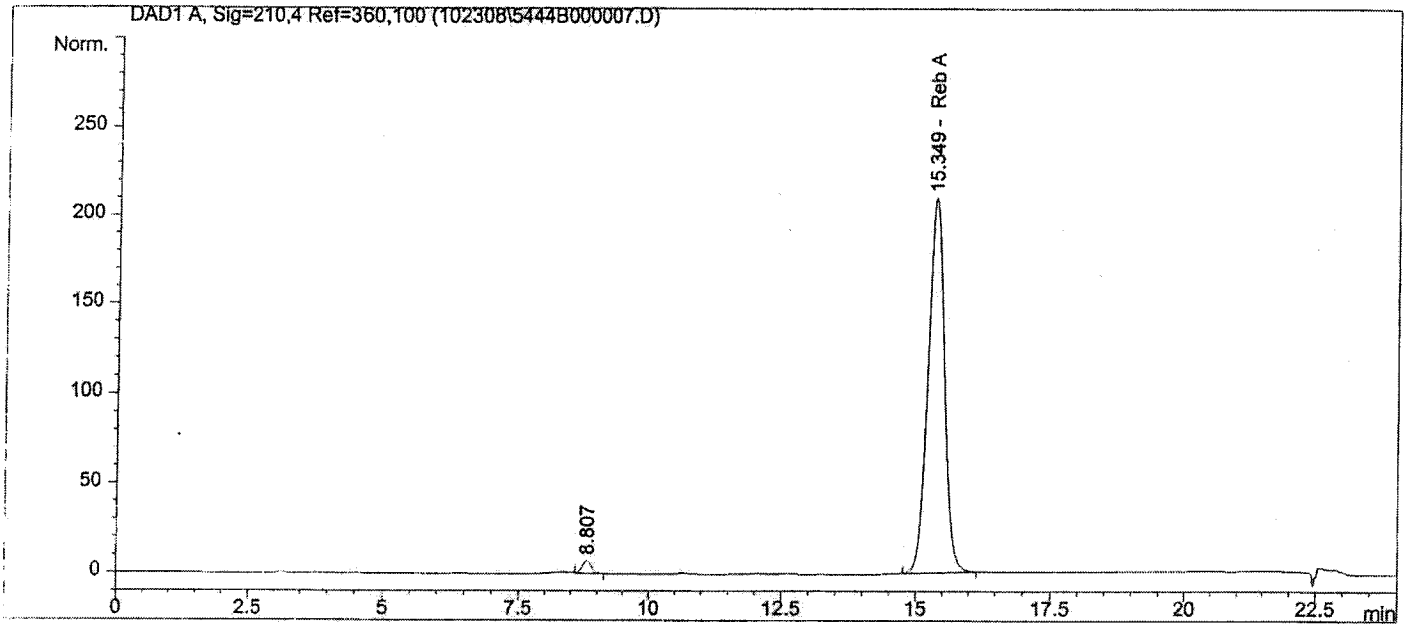
Warning : Calibrated compound(s) not found

*** End of Report ***

000262

Data File F:\HPLC10\DATA\102308\5444B000007.D
Sample Name: Accuracy

Injection Date	: 10/23/2008 6:16:08 PM	Seq. Line	: 4
Sample Name	: Accuracy	Location	: P1-F-03
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/23/2008 12:23:26 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/24/2008 9:29:00 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : Friday, October 24, 2008 9:24:38 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.94000 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.644		-	-	-		Stevioside
15.349	BB	4538.85791	4.31938e-4	101.057024		Reb A
Totals :				101.057024		

1 Warnings or Errors :

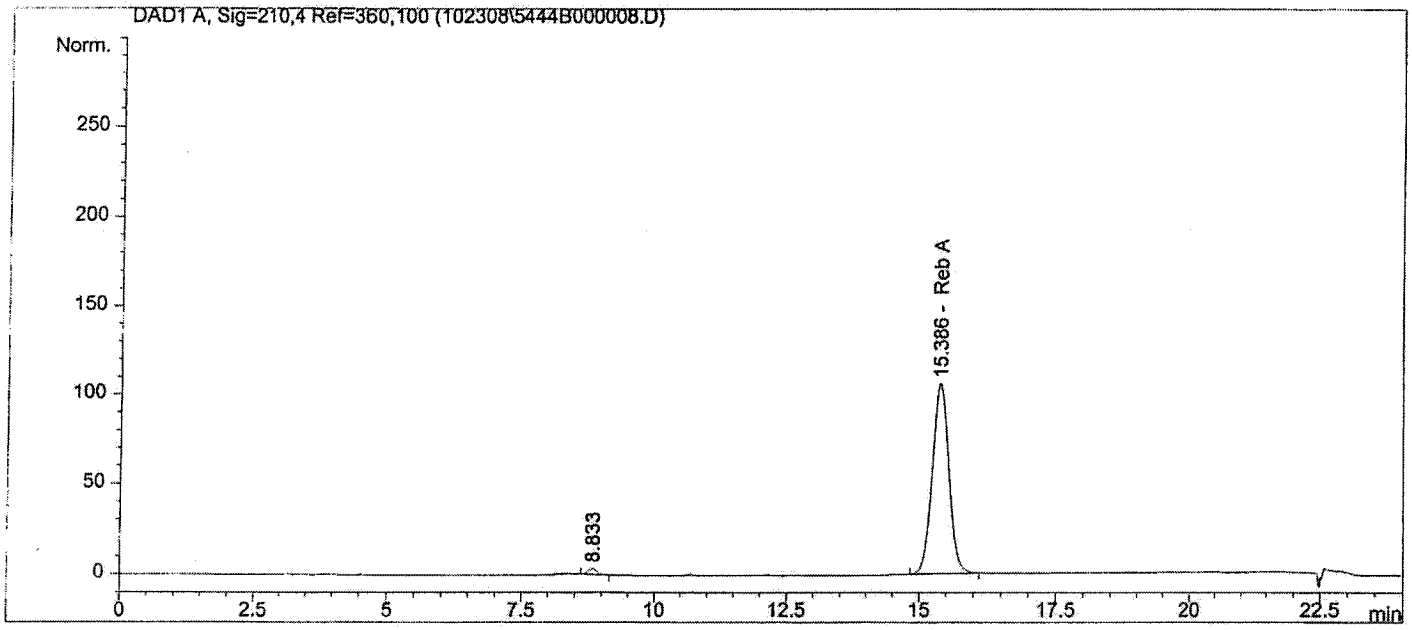
Warning : Calibrated compound(s) not found

*** End of Report ***

000263

Data File I:\HPLC10\DATA\102308\5444B000008.D
Sample Name: Accuracy 1:2

Injection Date	: 10/23/2008 6:51:02 PM	Seq. Line	: 5
Sample Name	: Accuracy 1:2	Location	: P1-F-04
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/23/2008 12:23:26 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/24/2008 9:29:29 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : Friday, October 24, 2008 9:24:38 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 9.69882e-1 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.644		-	-	-		Stevioside
15.386	BB	2288.80078	4.31938e-4	101.932046		Reb A

Totals : 101.932046

1 Warnings or Errors :

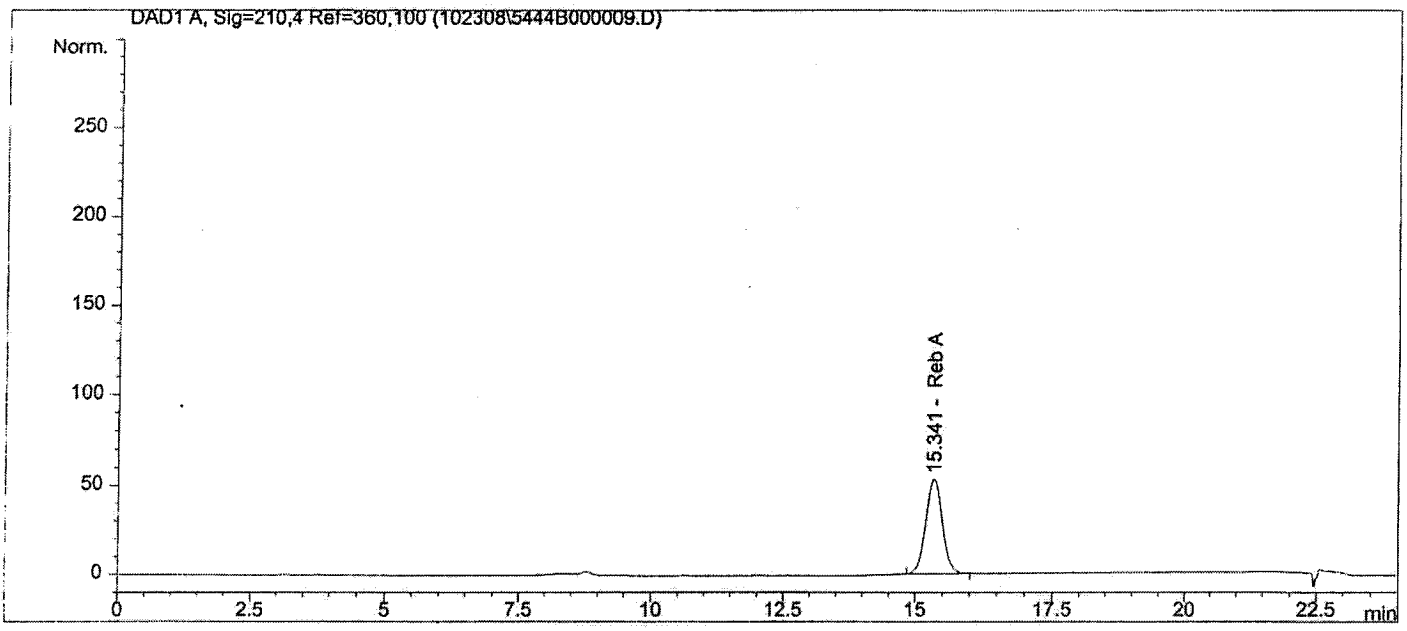
Warning : Calibrated compound(s) not found

*** End of Report ***

000264

Data File F:\HPLC10\DATA\102308\5444B000009.D
Sample Name: Accuracy 1:4

Injection Date	: 10/23/2008 7:25:54 PM	Seq. Line	: 6
Sample Name	: Accuracy 1:4	Location	: P1-F-05
Acq. Operator	: mze	Inj	: 1
Acq. Instrument	: HPLC10	Inj Volume	: 5 µl
Acq. Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/23/2008 12:23:26 PM by mze		
Analysis Method	: F:\HPLC10\METHODS\STEVIA.M		
Last changed	: 10/24/2008 9:29:50 AM by mze (modified after loading)		



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : Friday, October 24, 2008 9:24:38 AM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 4.84941e-1 [mg/ml]
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=360,100

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
10.644		-	-	-		Stevioside
15.341	BB	1143.76636	4.31938e-4	101.875572		Reb A

Totals : 101.875572

1 Warnings or Errors :

Warning : Calibrated compound(s) not found

*** End of Report ***

000265

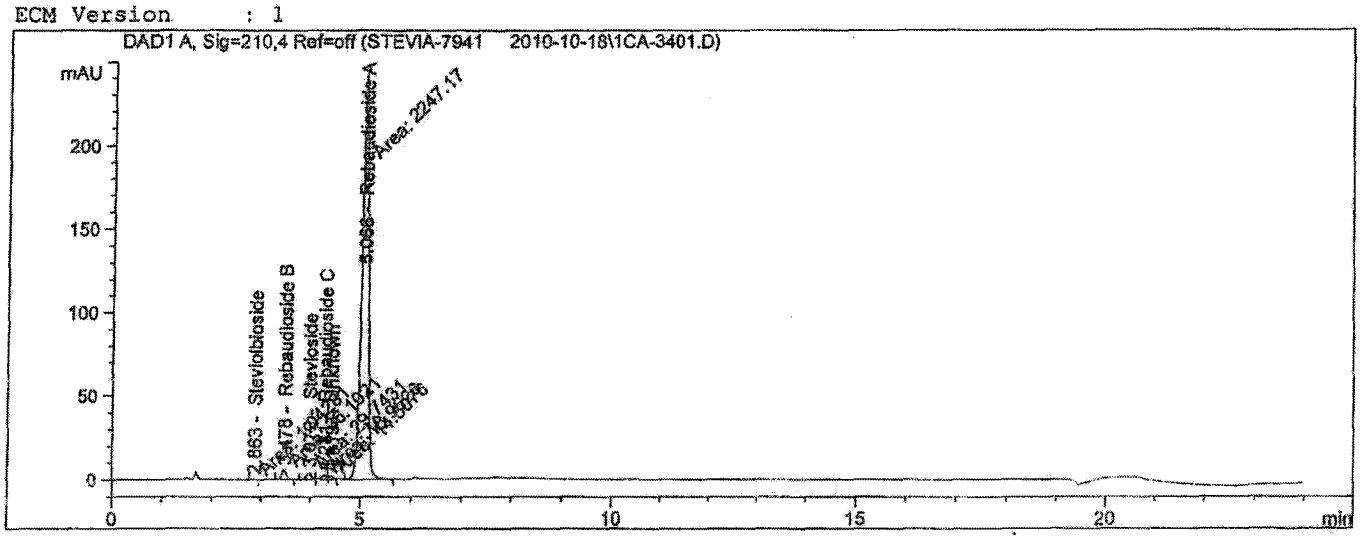
Appendix #10

Repeatability, Precision, Results (Sample Chromatograms)

Sample Name: 10-7947A

```
Acq. Operator    : (b) (6)                          Seq. Line :  34
Acq. Instrument  : HPLC 10                           Location : P1-C-01
Injection Date   : 10/19/2010 7:08:59 PM                 Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.02850  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.863	MM	1.04281	5.83724e-4	3.00079e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.478	MF	36.10210	7.34077e-4	1.306469		Rebaudioside B
3.979	MF	29.74307	7.34077e-4	1.076347		Stevioside
4.281	MF	18.96264	8.66741e-4	0.810239		Rebaudioside C
4.419	MF	14.50762	8.84430e-4	0.632535		Unknown
5.066	FM	2247.16724	8.84433e-4	97.977290		Rebaudioside A
Totals :				101.832889		

Sample Name: 10-7947A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

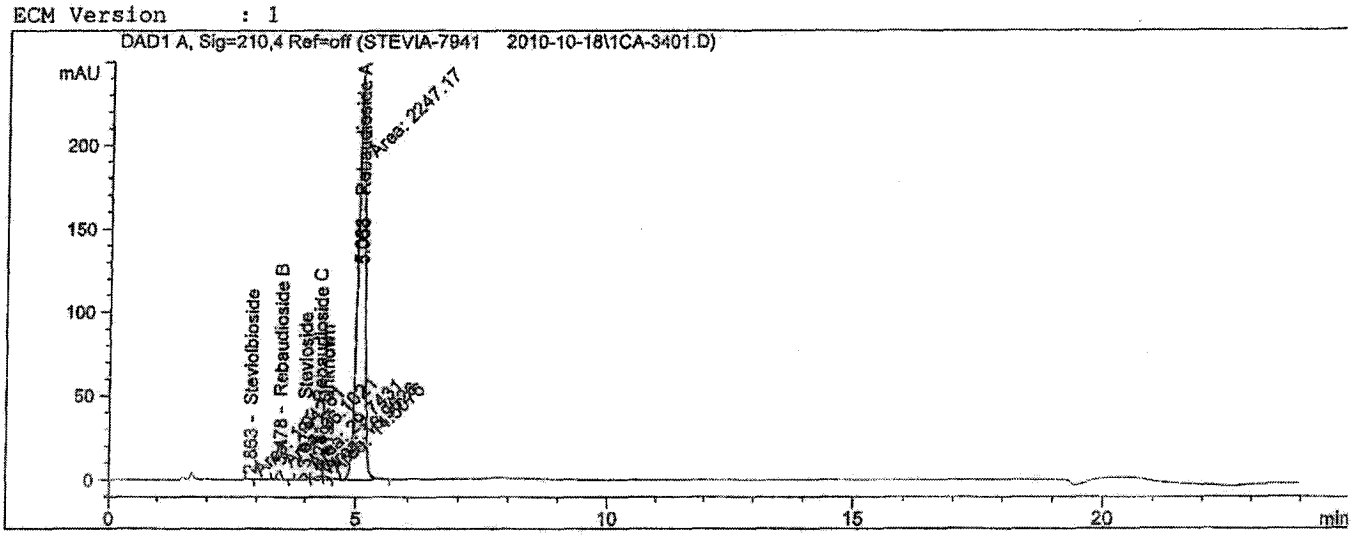
==========

*** End of Report ***

Sample Name: 10-7947A

```
Acq. Operator    : (b) (6)                          Seq. Line :  34
Acq. Instrument  : HPLC 10                           Location : P1-C-01
Injection Date   : 10/19/2010 7:08:59 PM                 Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:56:25 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.02850  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.863	-	1	3.00079e-2	1.13	0.1040	4200	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.478	-	1	1.306469	1.02	0.0947	7476	3.63	Rebaudioside B
3.979	-	1	1.076347	1.04	0.1086	7440	2.90	Stevioside
4.281	-	1	0.810239	1.33	0.1493	4553	1.38	Rebaudioside C
4.419	-	1	0.632535	0.76	0.1324	6173	0.58	Unknown
5.066	-	1	97.977290	1.05	0.1378	7491	2.82	Rebaudioside A

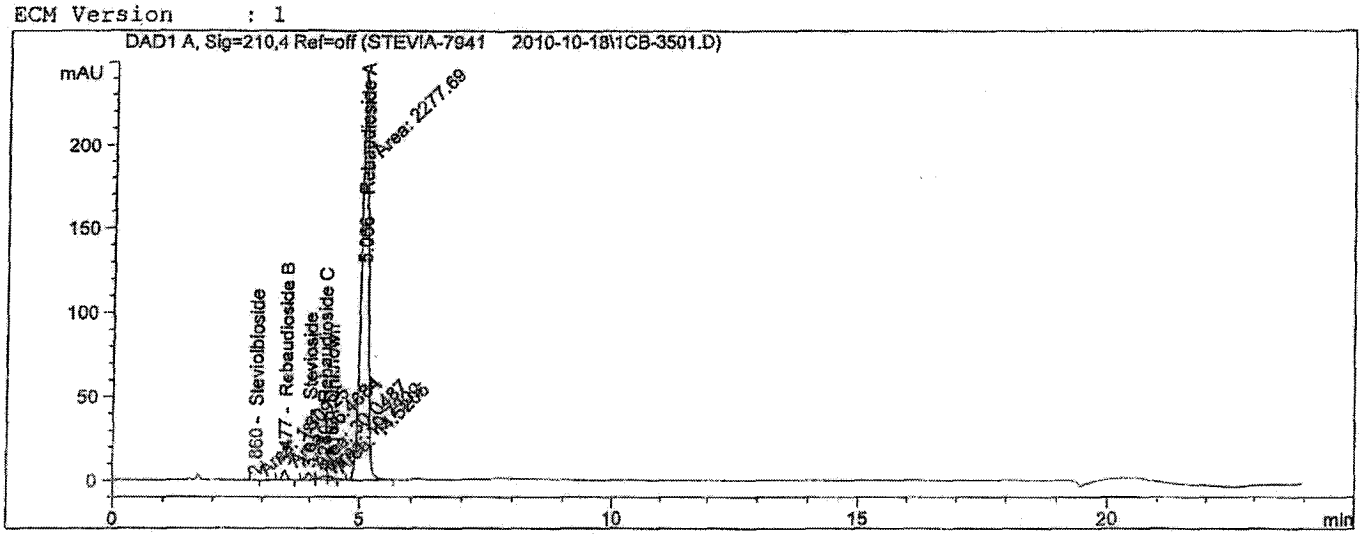
Sample Name: 10-7947A

*** End of Report ***

Sample Name: 10-7947B

```
Acq. Operator     : (b) (6)                          Seq. Line :  35
Acq. Instrument   : HPLC 10                           Location : P1-C-02
Injection Date    : 10/19/2010 7:48:42 PM                 Inj :   1
                                                    Inj Volume : 5.0 µl
Acq. Method       : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method   : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info       : Steviol Glycosides by HPLC (modified JECFA)

ECM Server        : http://us05sqlc/ecmwg
ECM Operator      : Mariel Esguerra
ECM Path          : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version       : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.06150  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.860	MM	1.30925	5.83724e-4	3.70720e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.477	MF	36.46843	7.34077e-4	1.298600		Rebaudioside B
3.978	MF	30.04865	7.34077e-4	1.069999		Stevioside
4.280	MF	19.73018	8.66741e-4	0.829539		Rebaudioside C
4.416	MF	14.52051	8.84430e-4	0.622963		Unknown
5.066	FM	2277.69287	8.84433e-4	97.718517		Rebaudioside A
Totals :				101.576689		

Sample Name: 10-7947B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

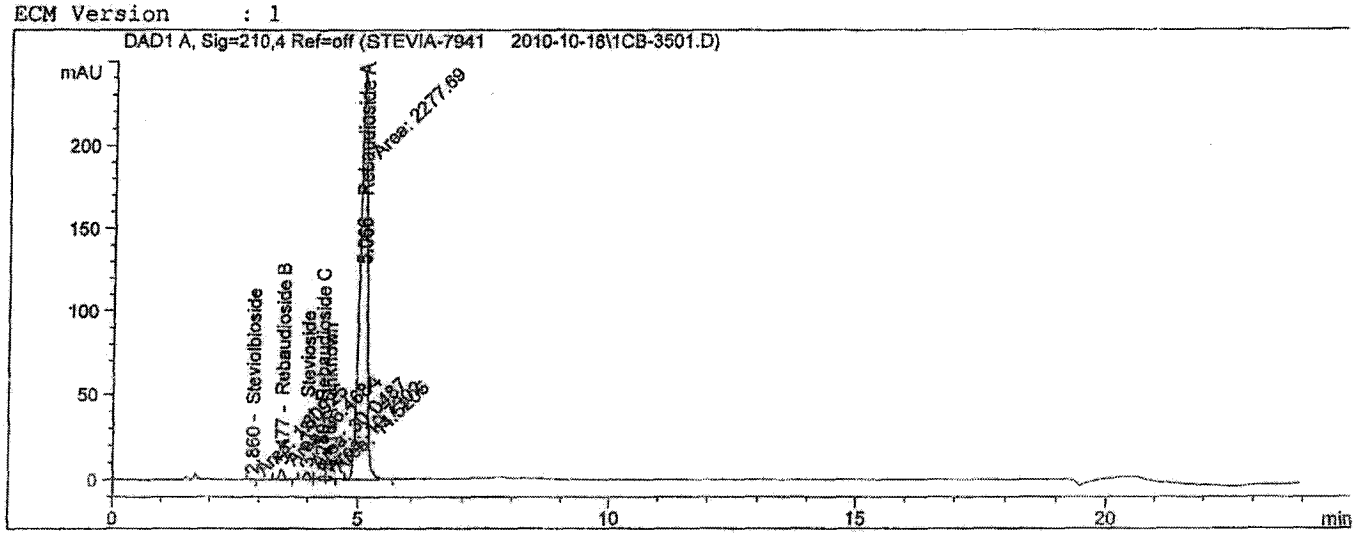
==========

*** End of Report ***

Sample Name: 10-7947B

```
Acq. Operator     : (b) (6)                          Seq. Line :  35
Acq. Instrument   : HPLC 10                           Location : P1-C-02
Injection Date    : 10/19/2010 7:48:42 PM                Inj :   1
                                                    Inj Volume : 5.0 µl
Acq. Method       : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method   : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 10/20/2010 3:56:25 PM by Mariel Esguerra
Method Info       : Steviol Glycosides by HPLC (modified JECFA)

ECM Server        : http://us05sqlc/ecmwg
ECM Operator      : Mariel Esguerra
ECM Path          : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version       : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :       10/20/2010 2:55:53 PM
Multiplier:                    :      1.0000
Dilution:                      :      1.0000
Sample Amount:                 :      2.06150  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resol ution	Name
2.860	-	1	3.70720e-2	1.11	0.1080	3885	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.477	-	1	1.298600	1.02	0.0947	7474	3.58	Rebaudioside B
3.978	-	1	1.069999	1.04	0.1095	7309	2.88	Stevioside
4.280	-	1	0.829539	1.26	0.1541	4275	1.34	Rebaudioside C
4.416	-	1	0.622963	0.65	0.1286	6537	0.57	Unknown
5.066	-	1	97.718517	1.05	0.1400	7253	2.84	Rebaudioside A

Sample Name: 10-7947B

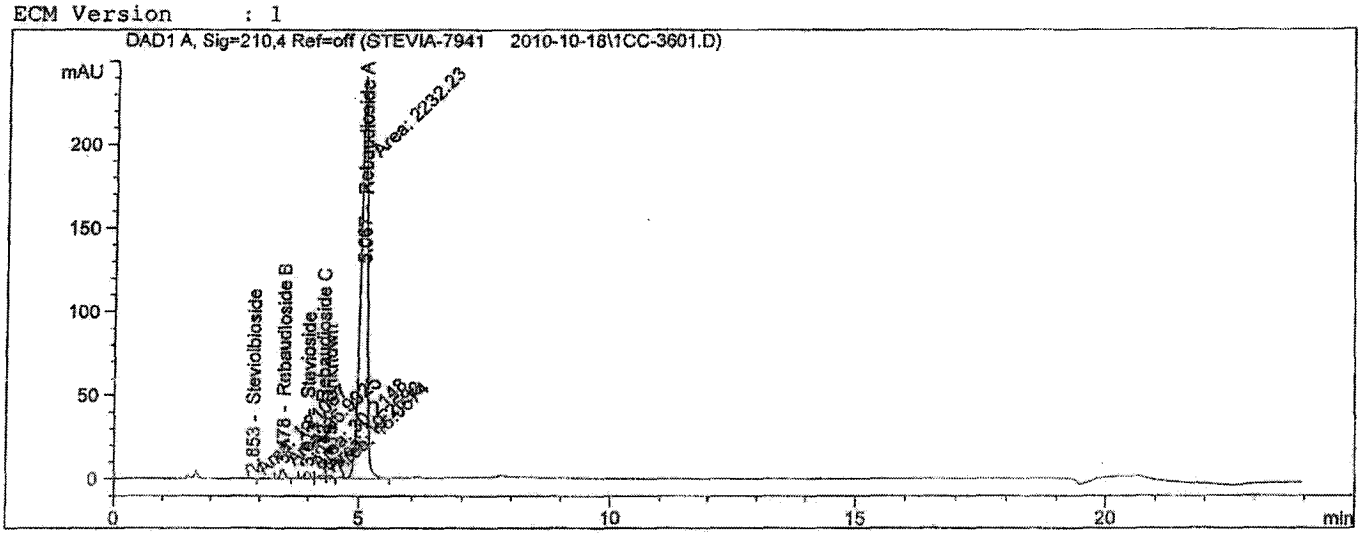
==========

*** End of Report ***

Sample Name: 10-7947C

```
Acq. Operator     : (b) (6)                    Seq. Line :  36
Acq. Instrument   : HPLC 10                     Location : P1-C-03
Injection Date    : 10/19/2010 8:28:27 PM            Inj :   1
                                               Inj Volume : 5.0 µl
Acq. Method       : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method   : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info       : Steviol Glycosides by HPLC (modified JECFA)

ECM Server        : http://us05sqlc/ecmwg
ECM Operator      : Mariel Esguerra
ECM Path          : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version       : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.02275  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.853	MM	1.21067	5.83724e-4	3.49374e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.478	MF	35.99249	7.34077e-4	1.306205		Rebaudioside B
3.979	MF	30.21482	7.34077e-4	1.096527		Stevioside
4.282	MF	19.26922	8.66741e-4	0.825679		Rebaudioside C
4.419	MF	15.05735	8.84430e-4	0.658370		Unknown
5.067	FM	2232.22656	8.84433e-4	97.602536		Rebaudioside A

Totals : 101.524254

000275

Sample Name: 10-7947C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

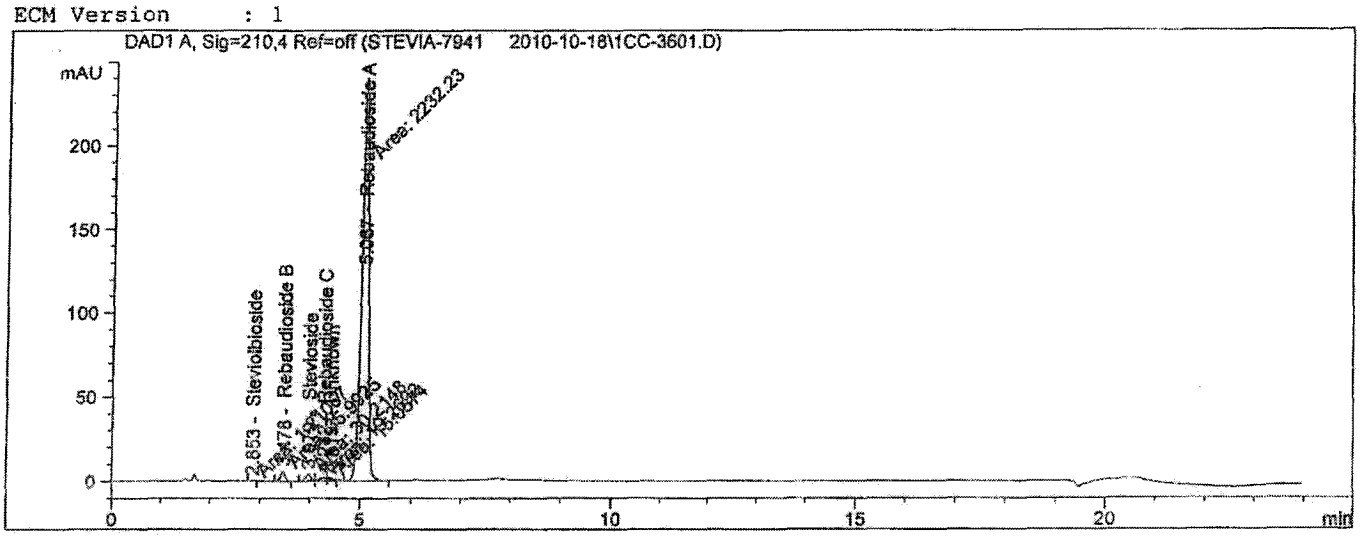
==========

*** End of Report ***

Sample Name: 10-7947C

```
Acq. Operator    : (b) (6)                    Seq. Line :  36
Acq. Instrument  : HPLC 10                     Location : P1-C-03
Injection Date   : 10/19/2010 8:28:27 PM            Inj :  1
                                              Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:56:25 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Sample Amount:               :      2.02275  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.853	-	1	3.49374e-2	0.90	0.1120	3596	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.478	-	1	1.306205	1.02	0.0947	7479	3.55	Rebaudioside B
3.979	-	1	1.096527	1.02	0.1095	7313	2.88	Stevioside
4.282	-	1	0.825679	1.35	0.1500	4514	1.37	Rebaudioside C
4.419	-	1	0.658370	0.74	0.1333	6086	0.57	Unknown
5.067	-	1	97.602536	1.05	0.1378	7494	2.81	Rebaudioside A

Sample Name: 10-7947C

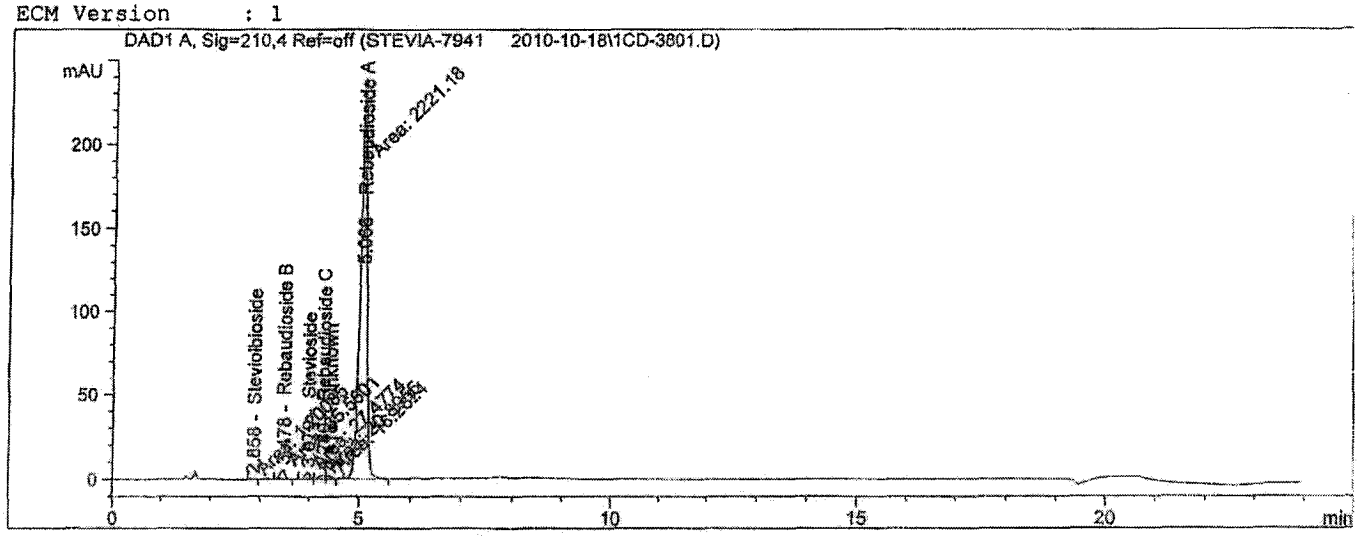
==========

*** End of Report ***

Sample Name: 10-7948A

```
Acq. Operator    : (b) (6)                          Seq. Line :  38
Acq. Instrument  : HPLC 10                           Location : P1-C-04
Injection Date   : 10/19/2010 9:47:54 PM                 Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.01475  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.858	MM	1.20085	5.83724e-4	3.47916e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.478	MF	35.56011	7.34077e-4	1.295638		Rebaudioside B
3.979	MF	27.47743	7.34077e-4	1.001144		Stevioside
4.280	MF	20.89553	8.66741e-4	0.898921		Rebaudioside C
4.418	MF	16.28239	8.84430e-4	0.714761		Unknown
5.066	FM	2221.17773	8.84433e-4	97.505068		Rebaudioside A

Totals : 101.450323

Sample Name: 10-7948A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

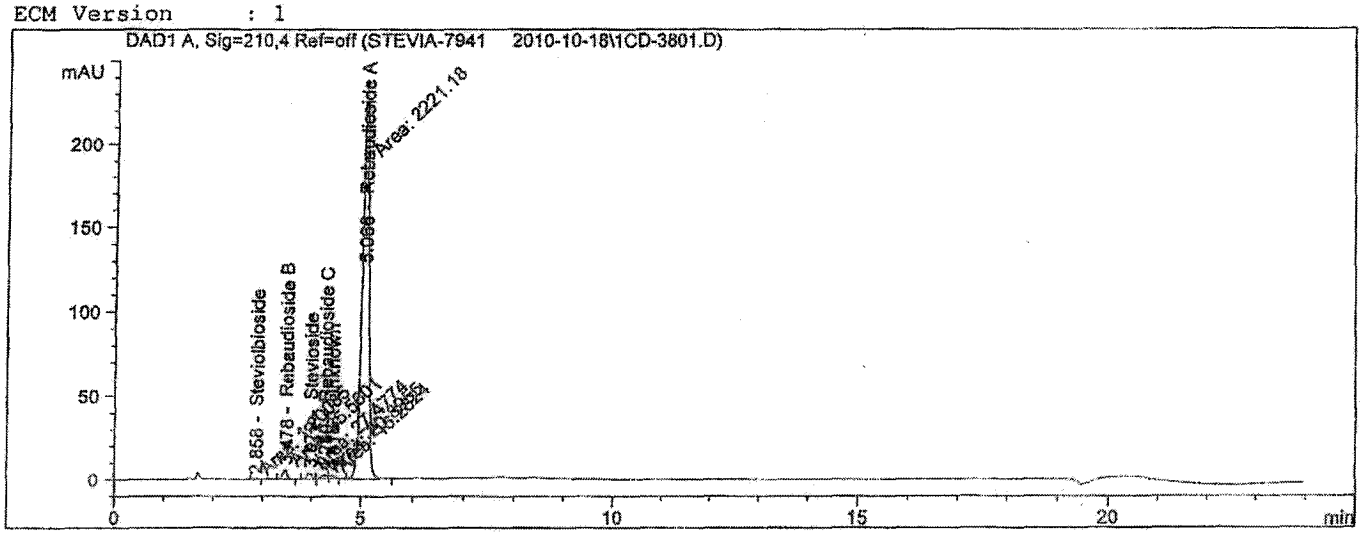
==========

*** End of Report ***

Sample Name: 10-7948A

```
Acq. Operator   : (b) (6)                          Seq. Line :  38
Acq. Instrument : HPLC 10                          Location : P1-C-04
Injection Date  : 10/19/2010 9:47:54 PM                 Inj :  1
                                                  Inj Volume : 5.0 µl
Acq. Method     : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed    : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info     : Steviol Glycosides by HPLC (modified JECFA)

ECM Server      : http://us05sqlc/ecmwg
ECM Operator    : Mariel Esguerra
ECM Path        : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version     : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Sample Amount:               :     2.01475  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.858	-	1	3.47916e-2	0.93	0.0973	4778	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.478	-	1	1.295638	1.03	0.0933	7692	3.82	Rebaudioside B
3.979	-	1	1.001144	1.03	0.1076	7574	2.93	Stevioside
4.280	-	1	0.898921	1.31	0.1489	4579	1.38	Rebaudioside C
4.418	-	1	0.714761	0.73	0.1305	6352	0.58	Unknown
5.066	-	1	97.505068	1.05	0.1378	7491	2.84	Rebaudioside A

Sample Name: 10-7948A

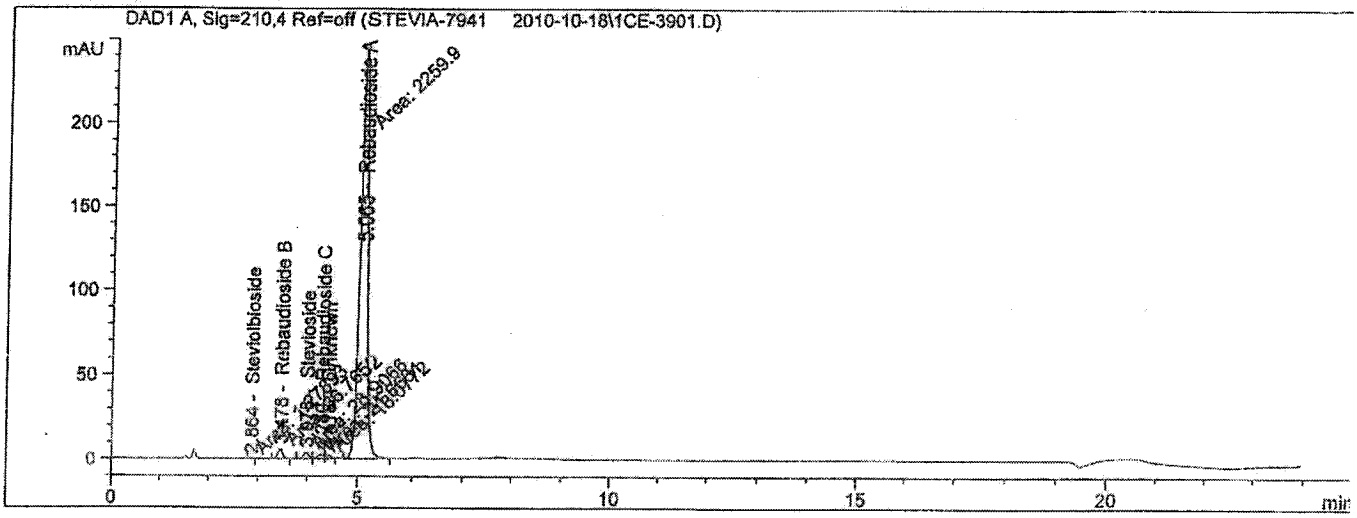
==========

*** End of Report ***

Sample Name: 10-7948B

```
Acq. Operator    : (b) (6)                          Seq. Line :  39
Acq. Instrument  : HPLC 10                           Location : P1-C-05
Injection Date   : 10/19/2010 10:27:39 PM                 Inj :  1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :      2.05850  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.864	FM	1.27893	5.83724e-4	3.62663e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.478	MF	36.76517	7.34077e-4	1.311074		Rebaudioside B
3.978	MF	28.90657	7.34077e-4	1.030831		Stevioside
4.280	MF	21.66306	8.66741e-4	0.912133		Rebaudioside C
4.418	FM	18.07721	8.84430e-4	0.776683		Unknown
5.065	FM	2259.89624	8.84433e-4	97.096298		Rebaudioside A

Totals : 101.163286

Sample Name: 10-7948B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

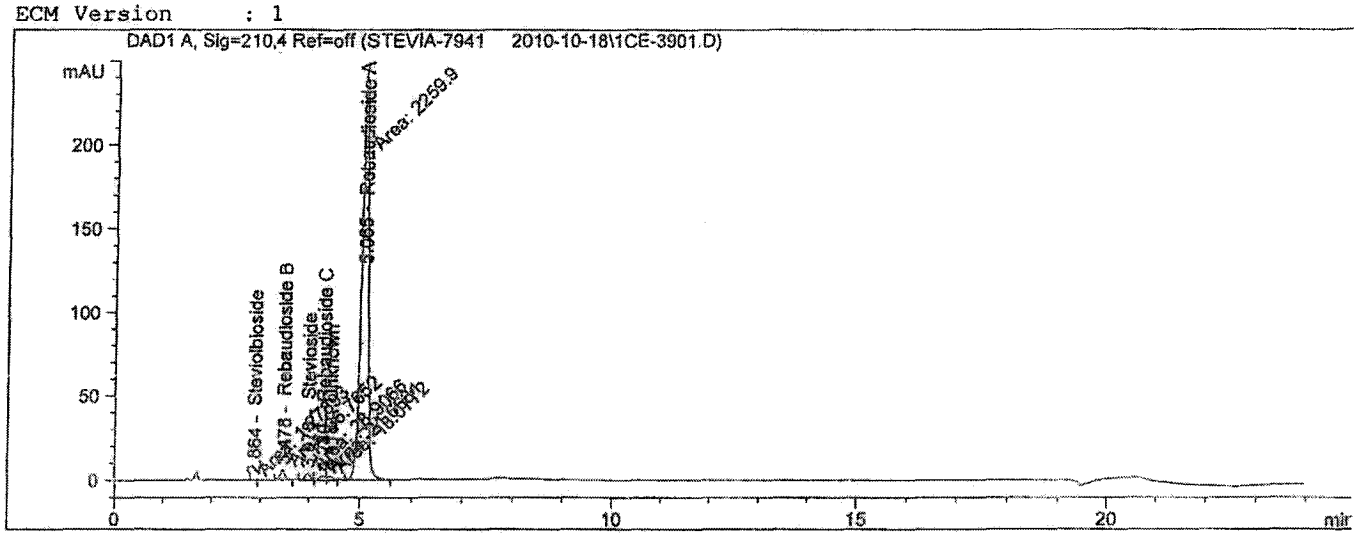
==========

*** End of Report ***

Sample Name: 10-7948B

Acq. Operator	: (b) (6)	Seq. Line	: 39
Acq. Instrument	: [illegible]	Location	: P1-C-05
Injection Date	: 10/19/2010 10:27:39 PM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 3:56:24 PM by Mariel Esguerra		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report with Performance

Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.05850 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resol ution	Name
2.864	-	1	3.62663e-2	0.96	0.1067	3995	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.478	-	1	1.311074	1.01	0.0947	7476	3.58	Rebaudioside B
3.978	-	1	1.030831	1.04	0.1095	7309	2.88	Stevioside
4.280	-	1	0.912133	1.46	0.1460	4761	1.39	Rebaudioside C
4.418	-	1	0.776683	0.75	0.1210	7391	0.61	Unknown
5.065	-	1	97.096298	1.05	0.1383	7427	2.93	Rebaudioside A

Sample Name: 10-7948B

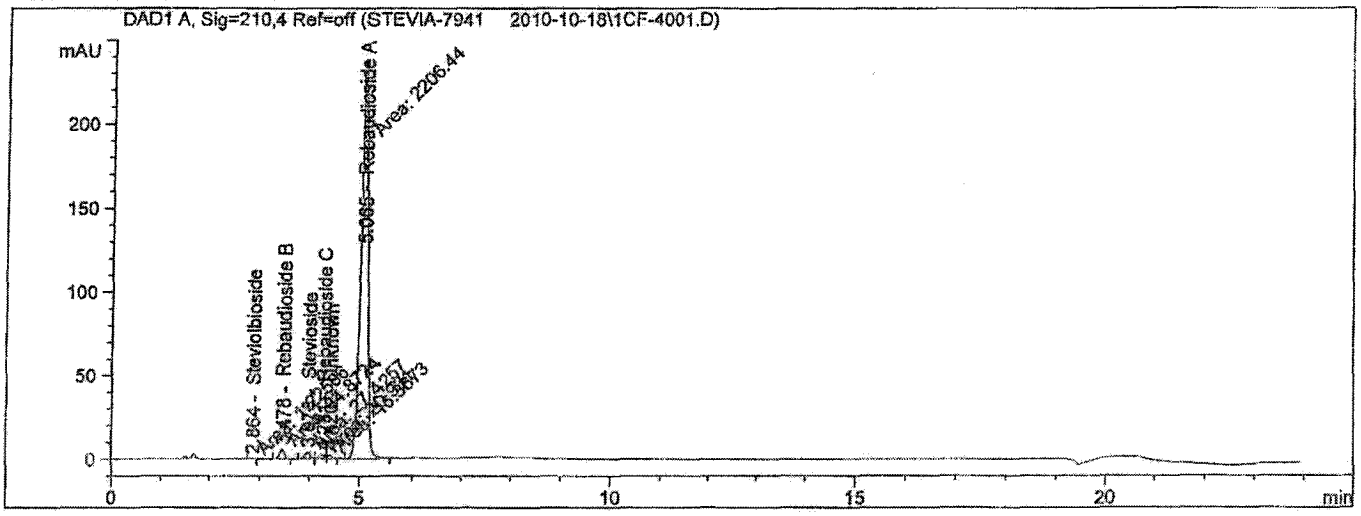
==========

*** End of Report ***

Sample Name: 10-7948C

```
Acq. Operator    : (b) (6)                    Seq. Line :  40
Acq. Instrument  : HPLC 10                     Location : P1-C-06
Injection Date   : 10/19/2010 11:07:23 PM          Inj :   1
                                             Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :      2.01150  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.864	MM	1.22506	5.83724e-4	3.55505e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.478	MF	34.87239	7.34077e-4	1.272633		Rebaudioside B
3.978	MF	27.42571	7.34077e-4	1.000874		Stevioside
4.281	MF	20.93103	8.66741e-4	0.901903		Rebaudioside C
4.420	MF	16.36725	8.84430e-4	0.719647		Unknown
5.065	FM	2206.43506	8.84433e-4	97.014389		Rebaudioside A
Totals :				100.944997		

Sample Name: 10-7948C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

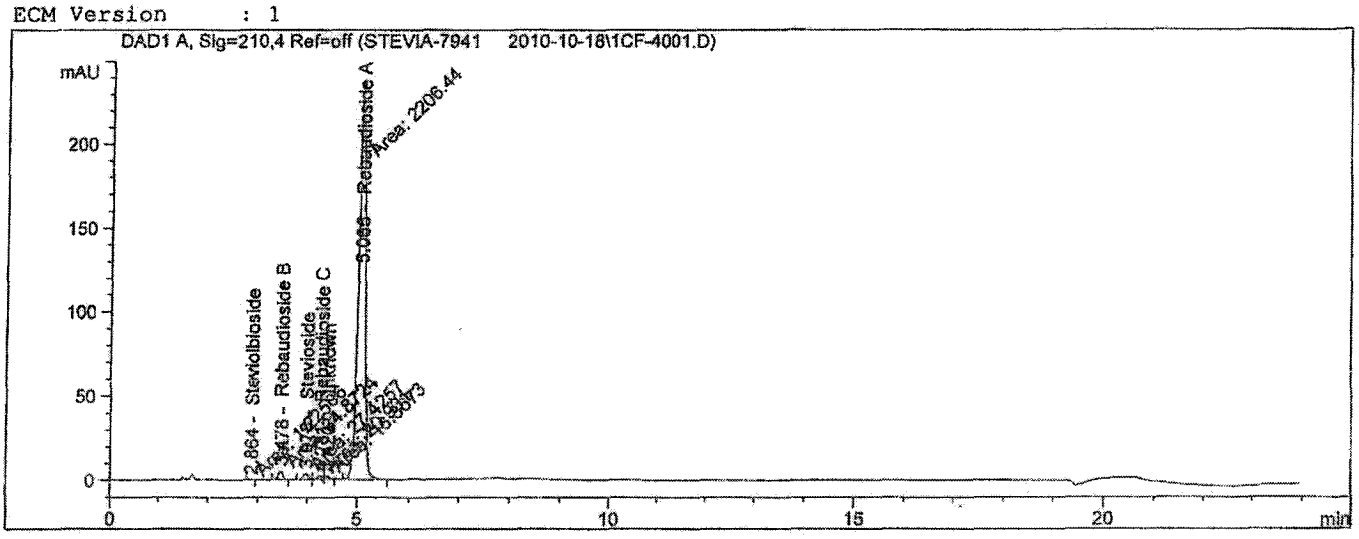
==========

*** End of Report ***

Sample Name: 10-7948C

```
Acq. Operator    : (b) (6)                          Seq. Line :  40
Acq. Instrument  : HPLC 10                           Location : P1-C-06
Injection Date   : 10/19/2010 11:07:23 PM                Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Sample Amount:               :     2.01150  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.864	-	1	3.55505e-2	1.04	0.1053	4097	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.478	-	1	1.272633	1.02	0.0947	7477	3.60	Rebaudioside B
3.978	-	1	1.000874	1.01	0.1086	7439	2.89	Stevioside
4.281	-	1	0.901903	1.31	0.1500	4512	1.37	Rebaudioside C
4.420	-	1	0.719647	0.73	0.1291	6489	0.58	Unknown
5.065	-	1	97.014389	1.05	0.1400	7252	2.82	Rebaudioside A

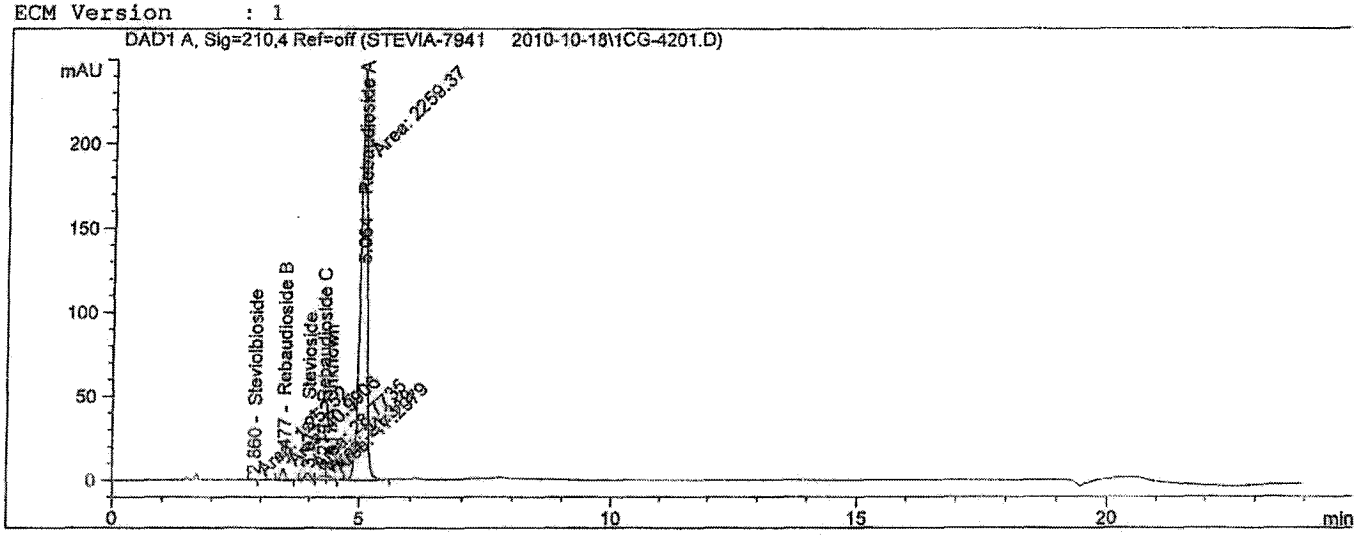
Sample Name: 10-7948C

*** End of Report ***

Sample Name: 10-7949A

```
Acq. Operator    : (b) (6)                          Seq. Line :  42
Acq. Instrument  : HPLC 10                           Location : P1-C-07
Injection Date   : 10/20/2010 12:26:51 AM                Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.04875  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.860	MM	1.25252	5.83724e-4	3.56864e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.477	MF	40.99055	7.34077e-4	1.468711		Rebaudioside B
3.978	MF	28.77353	7.34077e-4	1.030969		Stevioside
4.279	MF	21.31896	8.66741e-4	0.901917		Rebaudioside C
4.417	MF	17.29792	8.84430e-4	0.746738		Unknown
5.064	FM	2259.36597	8.84433e-4	97.535487		Rebaudioside A

Totals : 101.719509

Sample Name: 10-7949A

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

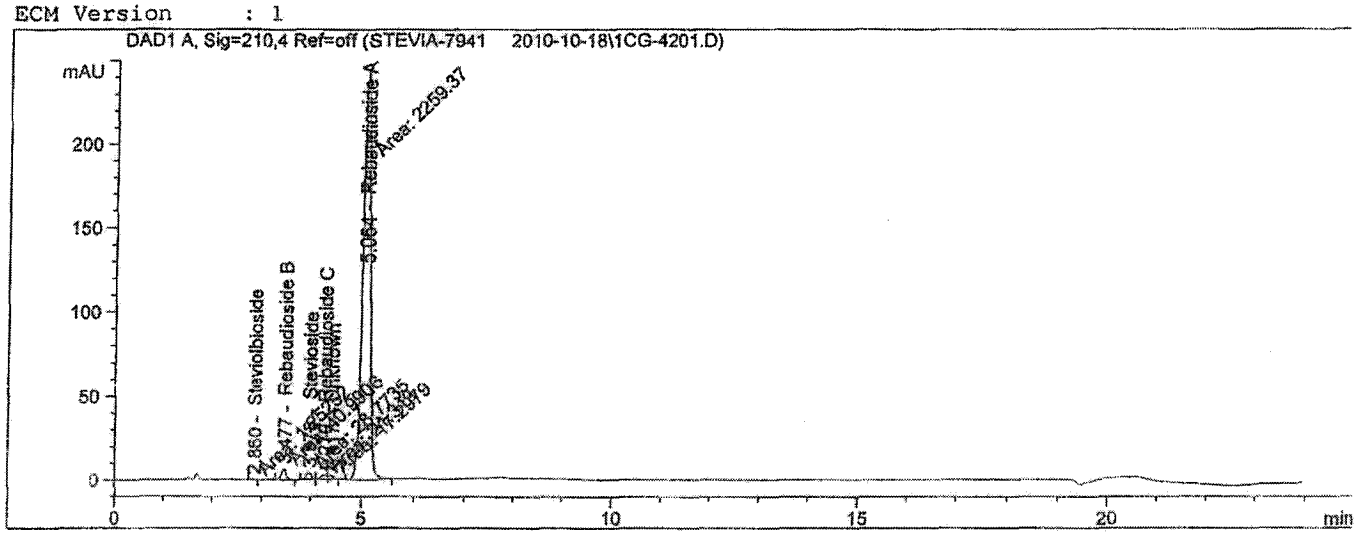
==========

*** End of Report ***

Sample Name: 10-7949A

```
Acq. Operator    : (b) (6)                          Seq. Line :  42
Acq. Instrument  : HPLC 10                           Location : P1-C-07
Injection Date   : 10/20/2010 12:26:51 AM                Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                 :      1.0000
Dilution:                   :      1.0000
Sample Amount:              :     2.04875  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.860	-	1	3.56864e-2	1.16	0.1107	3700	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.477	-	1	1.468711	1.00	0.0947	7472	3.53	Rebaudioside B
3.978	-	1	1.030969	1.01	0.1086	7436	2.90	Stevioside
4.279	-	1	0.901917	1.35	0.1493	4549	1.37	Rebaudioside C
4.417	-	1	0.746738	0.71	0.1343	5994	0.57	Unknown
5.064	-	1	97.535487	1.05	0.1378	7484	2.79	Rebaudioside A

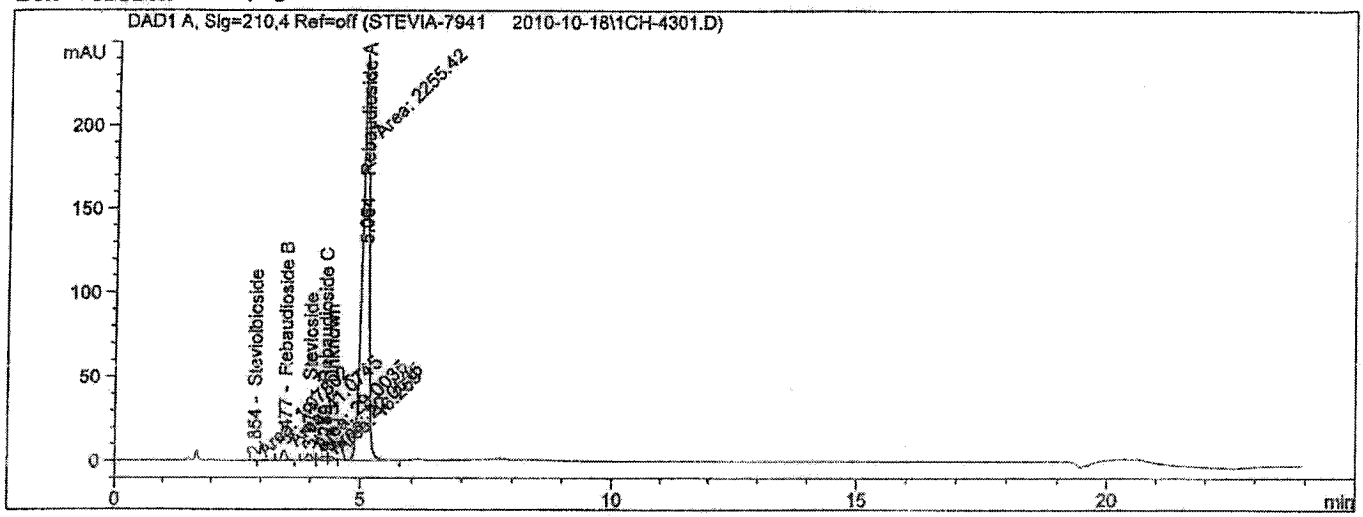
Sample Name: 10-7949A

*** End of Report ***

Sample Name: 10-7949B

```
Acq. Operator    : (b) (6)                    Seq. Line :  43
Acq. Instrument  : HPLC 10                     Location : P1-C-08
Injection Date   : 10/20/2010 1:06:36 AM            Inj :   1
                                              Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.05425  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.854	MM	1.07802	5.83724e-4	3.06324e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.477	MF	41.07445	7.34077e-4	1.467777		Rebaudioside B
3.979	MF	29.00351	7.34077e-4	1.036428		Stevioside
4.280	MF	22.05348	8.66741e-4	0.930493		Rebaudioside C
4.415	MF	16.25901	8.84430e-4	0.700010		Unknown
5.064	MM	2255.41528	8.84433e-4	97.104256		Rebaudioside A

Totals : 101.269596

Sample Name: 10-7949B

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

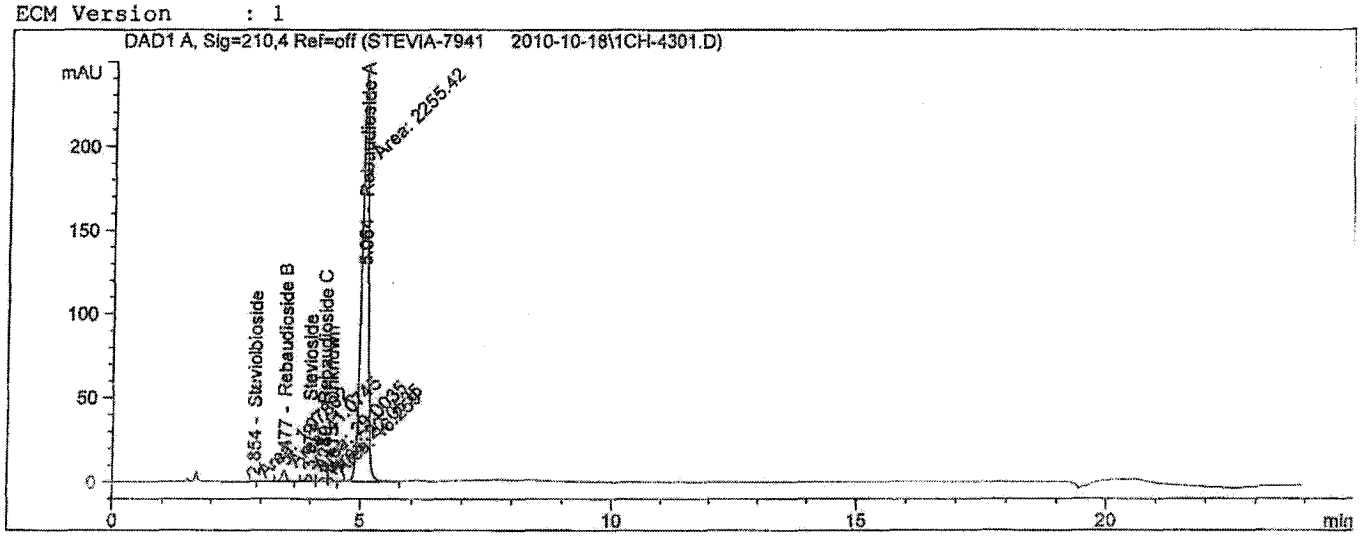
==========

*** End of Report ***

Sample Name: 10-7949B

```
Acq. Operator    : (b) (6)                    Seq. Line :  43
Acq. Instrument  : HPLC 10                     Location : P1-C-08
Injection Date   : 10/20/2010 1:06:36 AM            Inj :  1
                                              Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed     : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC (modified JECFA)

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version      : 1
```



ESTD Percent Report with Performance

```
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Sample Amount:               :     2.05425  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.854	-	1	3.06324e-2	0.86	0.1093	3774	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.477	-	1	1.467777	1.01	0.0947	7474	3.59	Rebaudioside B
3.979	-	1	1.036428	1.03	0.1095	7311	2.89	Stevioside
4.280	-	1	0.930493	1.27	0.1573	4101	1.33	Rebaudioside C
4.415	-	1	0.700010	0.62	0.1273	6667	0.56	Unknown
5.064	-	1	97.104256	1.05	0.1378	7485	2.88	Rebaudioside A

Sample Name: 10-7949B

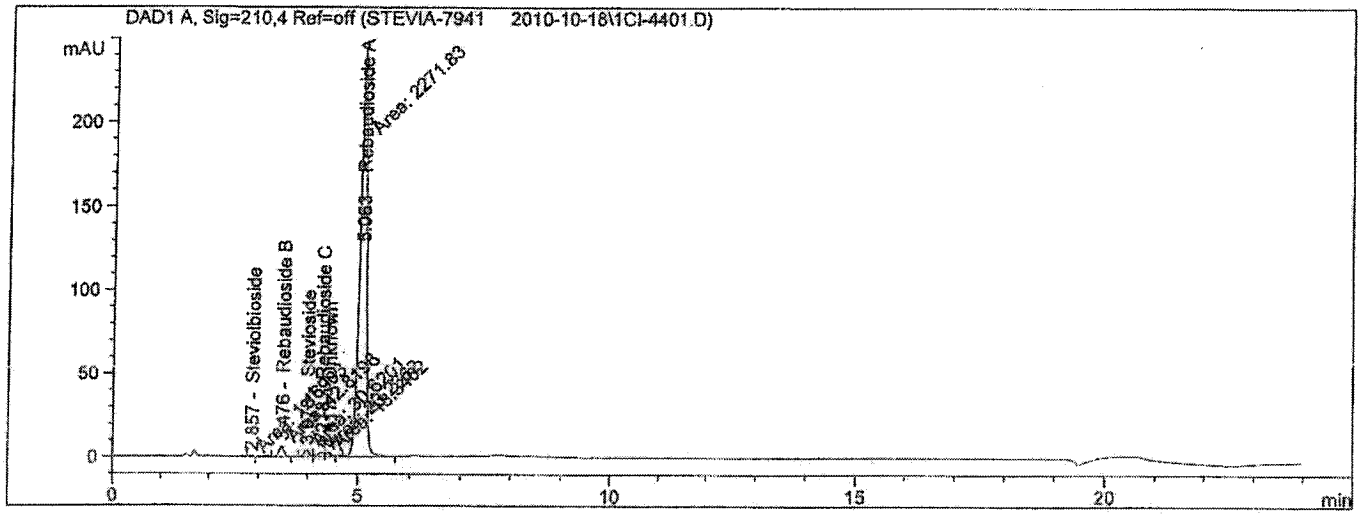
==========

*** End of Report ***

Sample Name: 10-7949C

```
Acq. Operator     : (b) (6)                          Seq. Line :  44
Acq. Instrument   : HPLC 10                          Location : P1-C-09
Injection Date    : 10/20/2010 1:46:20 AM                 Inj :   1
                                                   Inj Volume : 5.0 µl
Acq. Method       : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method   : C:\CHEM32\10\DATA\STEVIA-7941     2010-10-18\STEVIA.M
Last changed      : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info       : Steviol Glycosides by HPLC (modified JECFA)

ECM Server        : http://us05sqlc/ecmwg
ECM Operator      : Mariel Esguerra
ECM Path          : Petaluma\LC\HPLC-10\Data\STEVIA-7941     2010-10-18.SC.SSIzip
ECM Version       : 1
```



ESTD Percent Report

```
Sorted By             :      Signal
Calib. Data Modified  :      10/20/2010 2:55:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     2.06950  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.857	MM	1.16903	5.83724e-4	3.29737e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.476	MF	42.81983	7.34077e-4	1.518872		Rebaudioside B
3.978	MF	30.62015	7.34077e-4	1.086134		Stevioside
4.278	MF	23.23621	8.66741e-4	0.973171		Rebaudioside C
4.417	MF	18.34624	8.84430e-4	0.784053		Unknown
5.063	MM	2271.82593	8.84433e-4	97.090036		Rebaudioside A
Totals :				101.485239		

Sample Name: 10-7949C

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

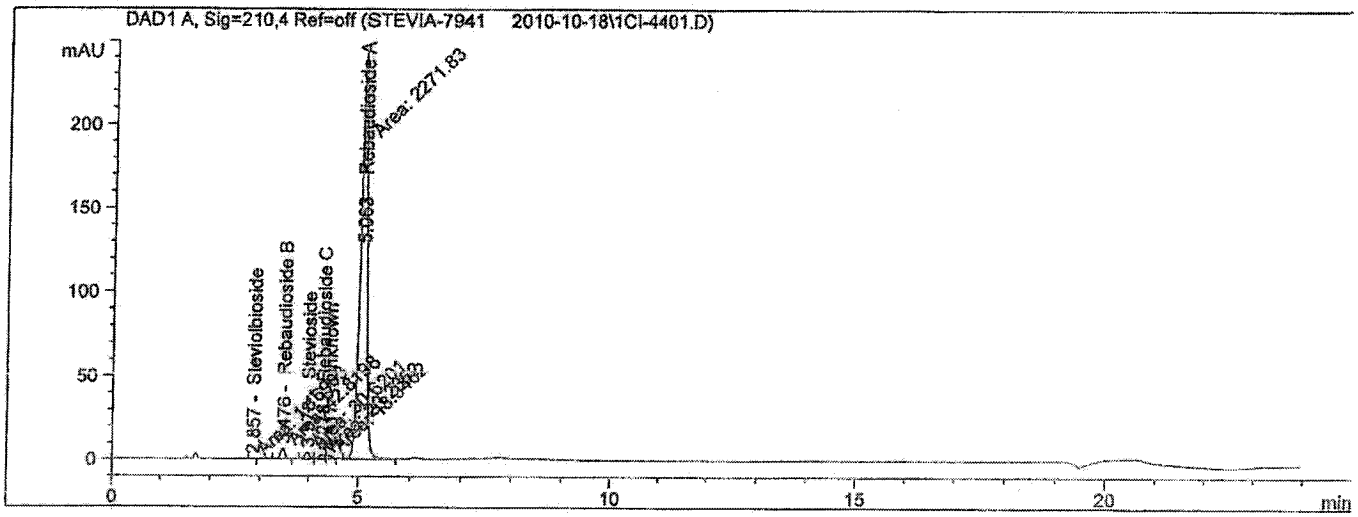
==========

*** End of Report ***

Sample Name: 10-7949C

Acq. Operator : (b) (6) Seq. Line : 44
Acq. Instrument : HPLC 10 Location : P1-C-09
Injection Date : 10/20/2010 1:46:20 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report with Performance

Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.06950 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.857	-	1	3.29737e-2	1.14	0.0967	4839	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.476	-	1	1.518872	1.01	0.0947	7470	3.80	Rebaudioside B
3.978	-	1	1.086134	1.04	0.1105	7182	2.87	Stevioside
4.278	-	1	0.973171	1.31	0.1580	4061	1.31	Rebaudioside C
4.417	-	1	0.784053	0.63	0.1341	6010	0.56	Unknown
5.063	-	1	97.090036	1.05	0.1378	7482	2.79	Rebaudioside A

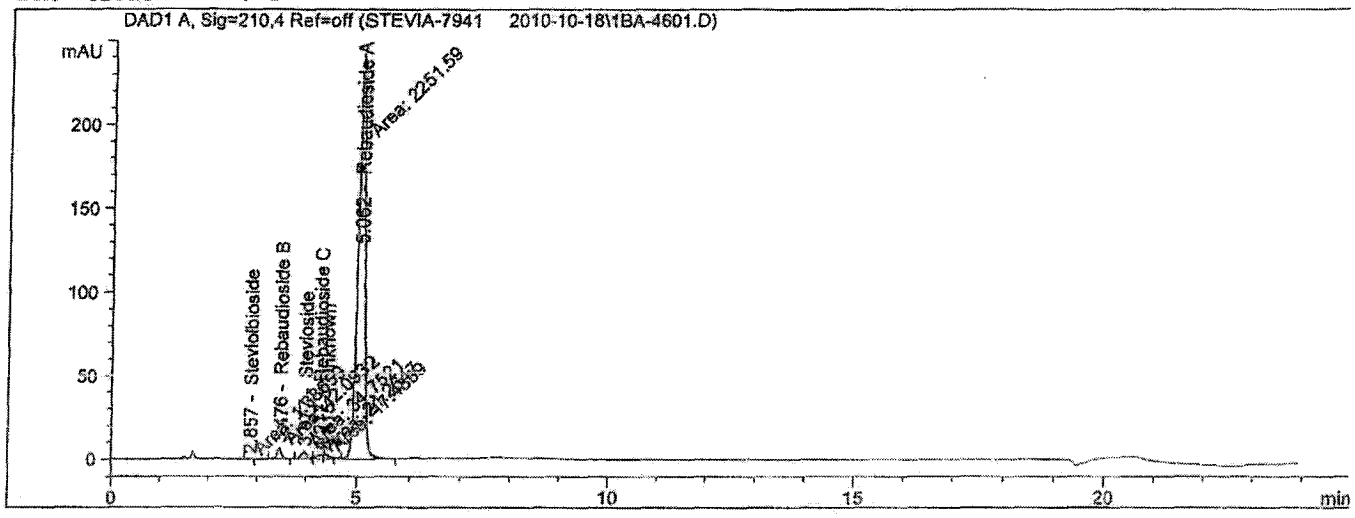
Sample Name: 10-7949C

*** End of Report ***

Sample Name: 10-7950A

Acq. Operator	: (b) (6)	Seq. Line	: 46
Acq. Instrument	: HPLC 10	Location	: P1-B-01
Injection Date	: 10/20/2010 3:05:48 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 9/24/2010 4:25:37 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M		
Last changed	: 10/20/2010 3:07:14 PM by Mariel Esguerra		
Method Info	: Steviol Glycosides by HPLC (modified JECFA)		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.04375 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.857	MM	1.38659	5.83724e-4	3.96028e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.476	MF	42.09318	7.34077e-4	1.511909		Rebaudioside B
3.977	MF	34.75207	7.34077e-4	1.248230		Stevioside
4.277	FM	21.26468	8.66741e-4	0.901821		Rebaudioside C
4.415	FM	17.43389	8.84430e-4	0.754449		Unknown
5.062	FM	2251.58691	8.84433e-4	97.437468		Rebaudioside A

Totals : 101.893479

2 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

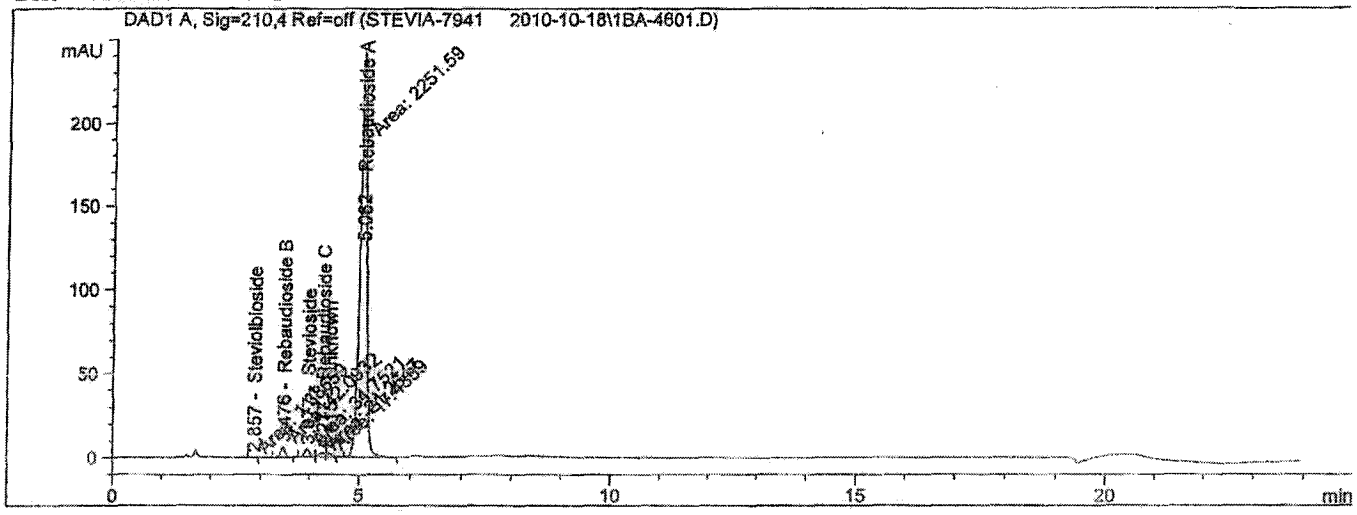
==========

*** End of Report ***

Sample Name: 10-7950A

Acq. Operator : (b) (6) Seq. Line : 46
Acq. Instrument : HPLC 10 Location : P1-B-01
Injection Date : 10/20/2010 3:05:48 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report with Performance

Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.04375 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.857	-	1	3.96028e-2	0.94	0.1240	2942	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.476	-	1	1.511909	1.01	0.0947	7468	3.32	Rebaudioside B
3.977	-	1	1.248230	0.00	0.1078	7543	2.91	Stevioside
4.277	-	1	0.901821	1.20	0.1491	4559	1.37	Rebaudioside C
4.415	-	1	0.754449	0.60	0.1248	6937	0.59	Unknown
5.062	-	1	97.437468	1.05	0.1400	7242	2.87	Rebaudioside A

Sample Name: 10-7950A

==========

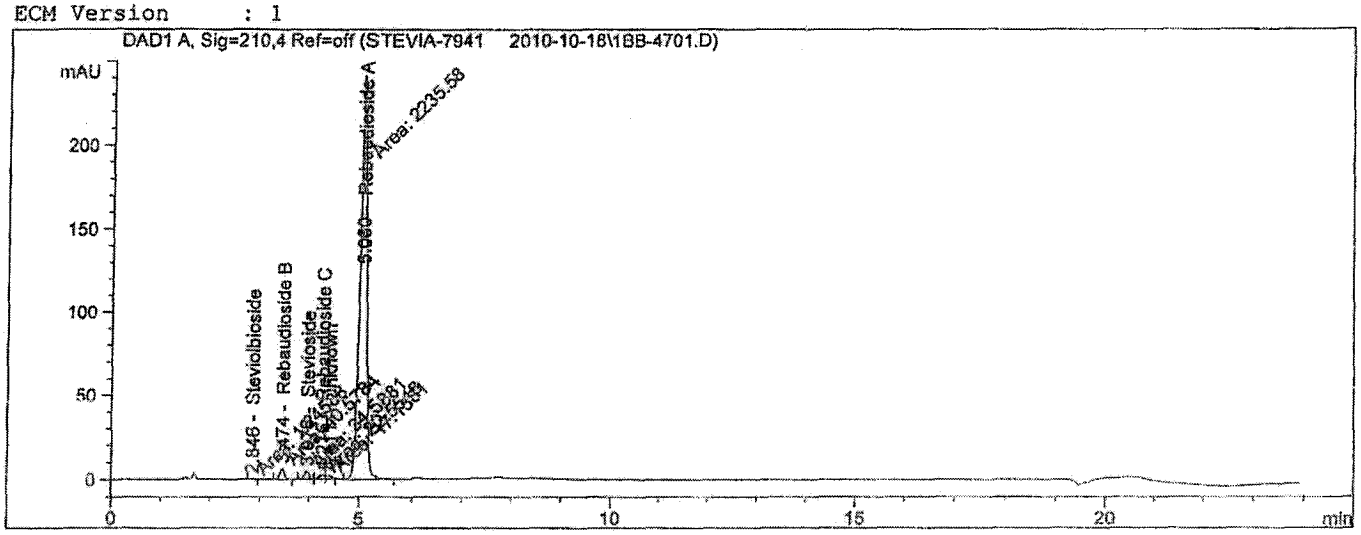
*** End of Report ***

Acq. Operator : (b) (6)
Acq. Instrument : HPLC 10
Injection Date : 10/20/2010 3:45:31 AM

Seq. Line : 47
Location : P1-B-02
Inj : 1
Inj Volume : 5.0 µl

Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.03350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.846	MM	1.24588	5.83724e-4	3.57636e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.474	MF	40.57844	7.34077e-4	1.464849		Rebaudioside B
3.976	MF	34.58813	7.34077e-4	1.248604		Stevioside
4.276	MF	22.55483	8.66741e-4	0.961357		Rebaudioside C
4.411	MF	17.15814	8.84430e-4	0.746259		Unknown
5.060	FM	2235.58105	8.84433e-4	97.232463		Rebaudioside A

Totals : 101.689295

000307

2 Warnings or Errors :

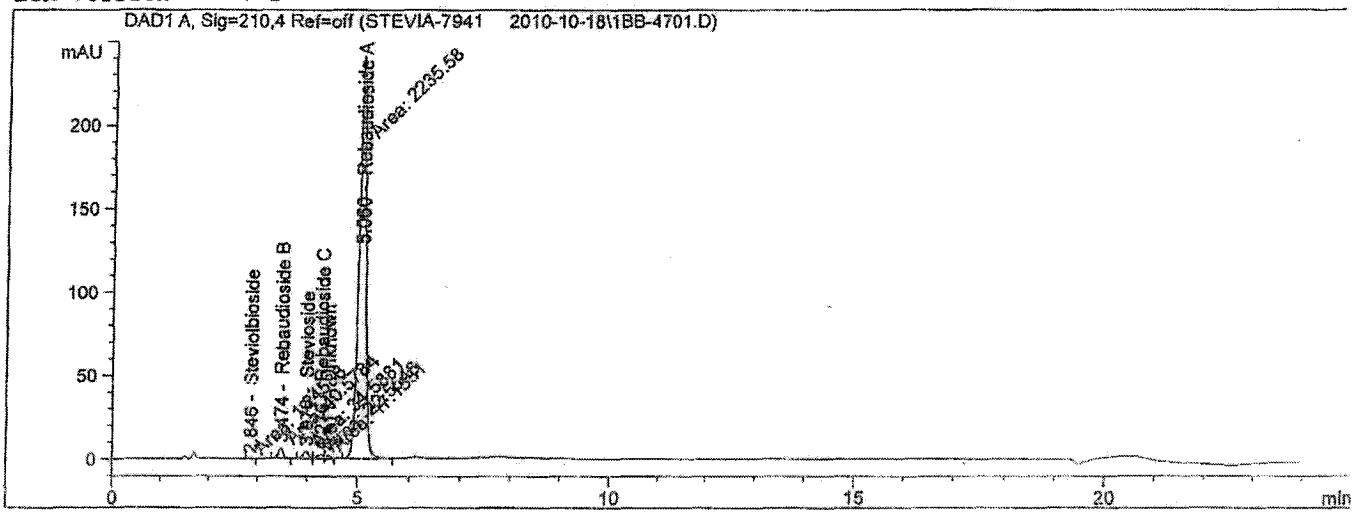
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Acq. Operator : (b) (6) Seq. Line : 47
Acq. Instrument : HPLC 10 Location : P1-B-02
Injection Date : 10/20/2010 3:45:31 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 3:56:24 PM by Mariel Esguerra
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report with Performance

Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.03350 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

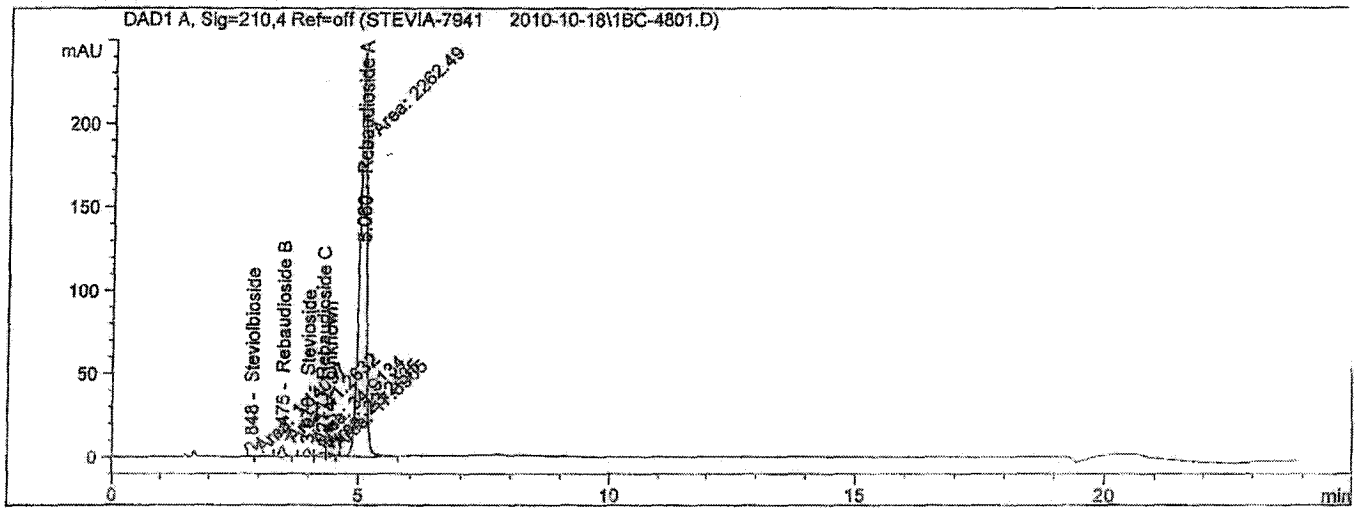
Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resol ution	Name
2.846	-	1	3.57636e-2	0.55	0.1120	3578	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.474	-	1	1.464849	1.01	0.0947	7462	3.57	Rebaudioside B
3.976	-	1	1.248604	1.02	0.1086	7428	2.90	Stevioside
4.276	-	1	0.961357	1.29	0.1525	4359	1.35	Rebaudioside C
4.411	-	1	0.746259	0.66	0.1305	6333	0.56	Unknown
5.060	-	1	97.232463	1.05	0.1378	7471	2.84	Rebaudioside A

*** End of Report ***

Acq. Operator : (b) (6) Seq. Line : 48
Acq. Instrument : HPLC 10 Location : P1-B-03
Injection Date : 10/20/2010 4:25:15 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/20/2010 3:07:14 PM by Mariel Esguerra
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.06075 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
2.848	MF	1.14097	5.83724e-4	3.23189e-2		Steviolbioside
3.195		-	-	-		Dulcoside A
3.475	MF	41.26323	7.34077e-4	1.469872		Rebaudioside B
3.976	MF	34.91344	7.34077e-4	1.243681		Stevioside
4.277	MF	23.24354	8.66741e-4	0.977611		Rebaudioside C
4.414	MF	17.69052	8.84430e-4	0.759239		Unknown
5.060	MM	2262.48560	8.84433e-4	97.101415		Rebaudioside A

Totals : 101.584137

2 Warnings or Errors :

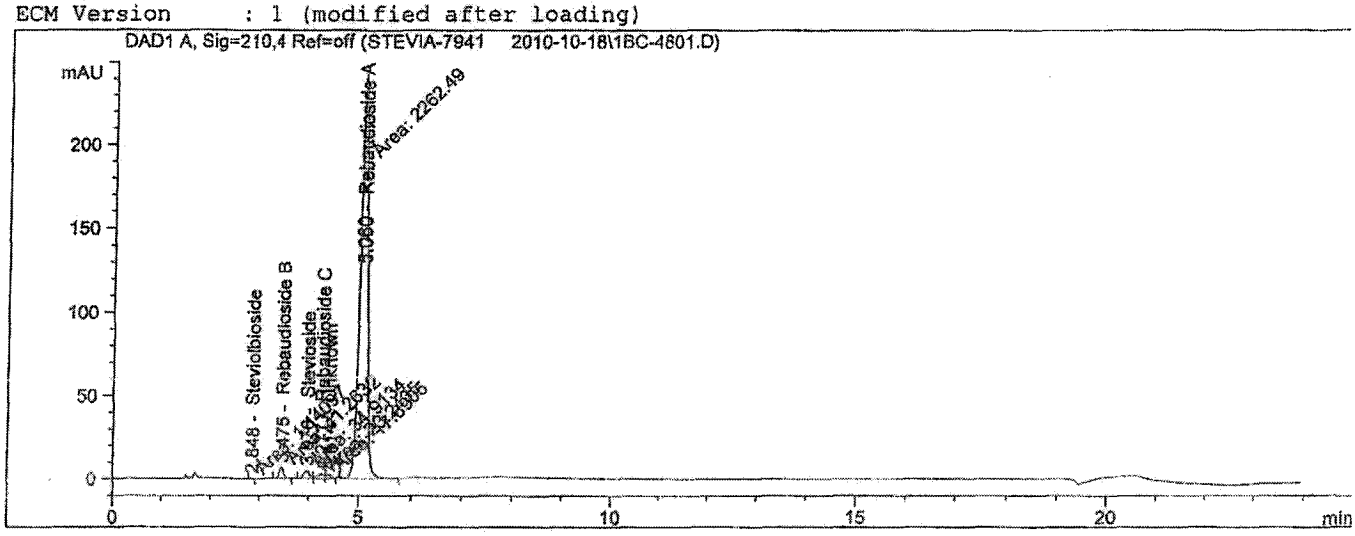
Warning : Calibration warnings (see calibration table listing)
Warning : Calibrated compound(s) not found

==========

*** End of Report ***

Acq. Operator : (b) (6) Seq. Line : 48
Acq. Instrument : HPLC 10 Location : P1-B-03
Injection Date : 10/20/2010 4:25:15 AM Inj : 1
Inj Volume : 5.0 µl
Acq. Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 9/24/2010 4:25:37 PM by Mariel Esguerra
Analysis Method : C:\CHEM32\10\DATA\STEVIA-7941 2010-10-18\STEVIA.M
Last changed : 10/21/2010 10:39:25 AM by Mariel Esguerra
(modified after loading)
Method Info : Steviol Glycosides by HPLC (modified JECFA)

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\STEVIA-7941 2010-10-18.SC.SSIzip
ECM Version : 1 (modified after loading)



ESTD Percent Report with Performance

Calib. Data Modified : 10/20/2010 2:55:53 PM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 2.06075 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	k'	Sig	Amount %	Symm.	Width [min]	Plates	Resolution	Name
2.848	-	1	3.23189e-2	0.74	0.1089	3790	-	Steviolbioside
3.195	-	1	not f	0.00	-	-	-	Dulcoside A
3.475	-	1	1.469872	1.00	0.0947	7464	3.62	Rebaudioside B
3.976	-	1	1.243681	1.01	0.1095	7300	2.88	Stevioside
4.277	-	1	0.977611	1.29	0.1533	4310	1.35	Rebaudioside C
4.414	-	1	0.759239	0.65	0.1296	6422	0.57	Unknown
5.060	-	1	97.101415	1.04	0.1400	7238	2.82	Rebaudioside A

*** End of Report ***

Appendix #11

Sample #10-7941 through 7945

Steviol Glycosides

Lot #s 100307, 100309, 100405, 100508, 100602

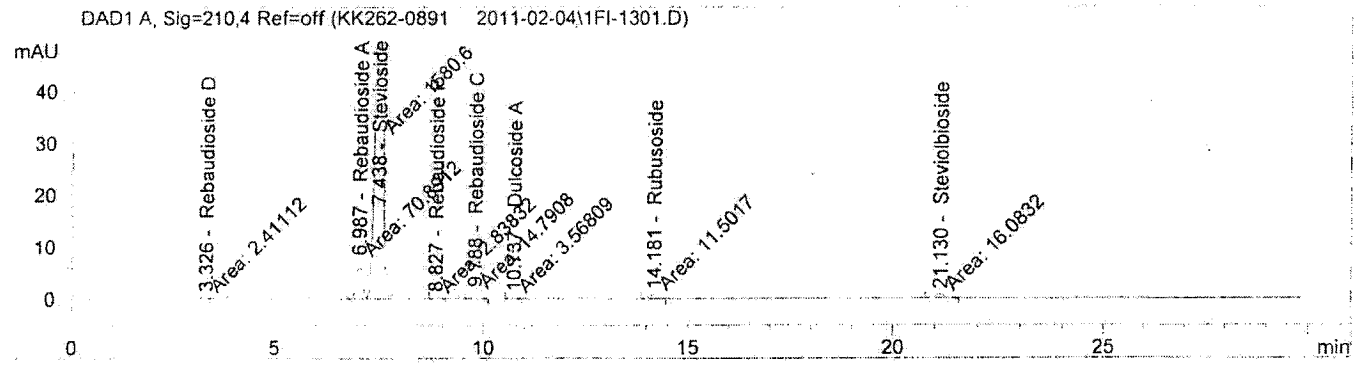
Rebaudioside D and F

Sample Chromatograms

Sample Name: 10-7941

Acq. Operator	: Mariel Esguerra	Seq. Line	: 13
Acq. Instrument	: HPLC 10	Location	: P1-F-09
Injection Date	: 2/5/2011 3:31:21 AM	Inj	: 1
		Inj Volume	: 5.0 µl
Acq. Method	: C:\CHEM32\10\DATA\KK262-0891 2011-02-04\KK262.M		
Last changed	: 2/4/2011 6:04:26 PM by Mariel Esguerra		
Analysis Method	: C:\CHEM32\17\DATA\KK262-0891 2011-02-04\KK262.M		
Last changed	: 3/18/2011 9:59:38 AM by Mariel Esguerra		
Method Info	: Steviol Glycosides by HPLC JECFA 2010		

ECM Server : http://us05sqlc/ecmwg
ECM Operator : Mariel Esguerra
ECM Path : Petaluma\LC\HPLC-10\Data\KK262-0891 2011-02-04.SC.SSIzip
ECM Version : 22



ESTD Percent Report

Sorted By : Signal
Calib. Data Modified : Friday, March 18, 2011 9:50:31 AM
Multiplier: : 1.0000
Dilution: : 1.0000
Sample Amount: : 1.25500 [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
3.326	FM	2.41112	1.05263e-3	0.202233		Rebaudioside D
6.987	MF	70.89122	8.95361e-4	5.057627		Rebaudioside A
7.438	FM	1580.59949	7.51883e-4	94.695310		Stevioside
8.827	FM	2.83832	8.72181e-4	0.197254		Rebaudioside F
9.788	MM	14.79082	8.87218e-4	1.045632		Rebaudioside C
10.737	MM	3.56809	7.36842e-4	0.209492		Dulcoside A
14.181	MM	11.50169	6.01504e-3	5.512601		Rubusoside
19.595		-	-	-		Rebaudioside B
21.130	MM	16.08315	6.01504e-4	0.770843		Steviolbioside

Totals : 107.690990

1 Warnings or Errors :

000316

Sample Name: 10-7941

Warning : Calibrated compound(s) not found

==========

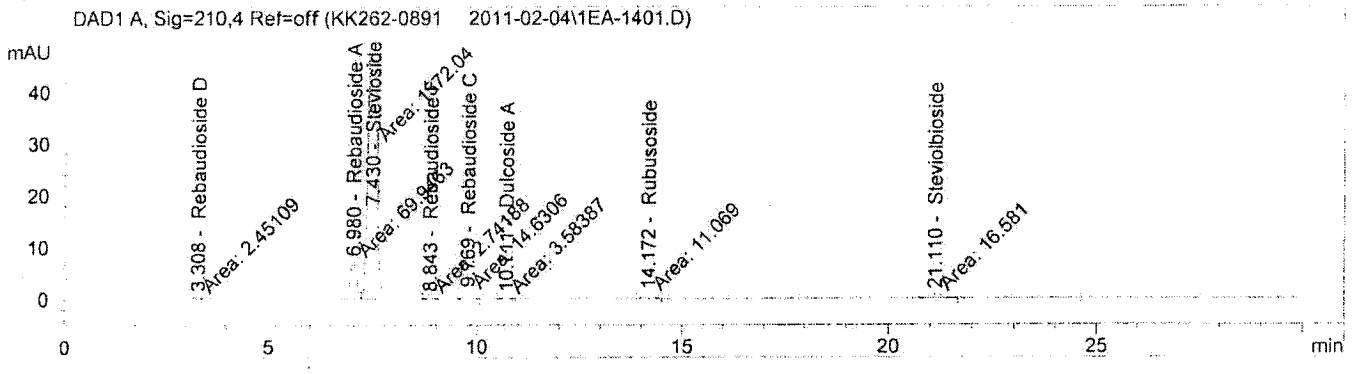
*** End of Report ***

000317

Sample Name: 10-7942

```
==========================================================================
Acq. Operator    : Mariel Esguerra                    Seq. Line :  14
Acq. Instrument  : HPLC 10                             Location : P1-E-01
Injection Date   : 2/5/2011 4:02:09 AM                     Inj :  1
                                                     Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\KK262-0891     2011-02-04\KK262.M
Last changed     : 2/4/2011 6:04:26 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\17\DATA\KK262-0891     2011-02-04\KK262.M
Last changed     : 3/18/2011 9:59:38 AM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC JECFA 2010

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\KK262-0891     2011-02-04.SC.SSIzip
ECM Version      : 22
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, March 18, 2011 9:50:31 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     1.25200  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [mAU*s]                   %
-------|------|----------|----------|----------|--|------------------
  3.308 MM       2.45109 1.05263e-3   0.206078    Rebaudioside D
  6.980 MF      69.94627 8.95361e-4   5.002168    Rebaudioside A
  7.430 FM    1572.04163 7.51883e-4  94.408277    Stevioside
  8.843 MF       2.74188 8.72181e-4   0.191008    Rebaudioside F
  9.769 MF      14.63060 8.87218e-4   1.036784    Rebaudioside C
 10.711 MM       3.58387 7.36842e-4   0.210922    Dulcoside A
 14.172 MM      11.06899 6.01504e-3   5.317925    Rubusoside
 19.595          -          -          -         Rebaudioside B
 21.110 MM      16.58103 6.01504e-4   0.796610    Steviolbioside

Totals :                             107.169771

1 Warnings or Errors :
```

000318

Sample Name: 10-7942

Warning : Calibrated compound(s) not found

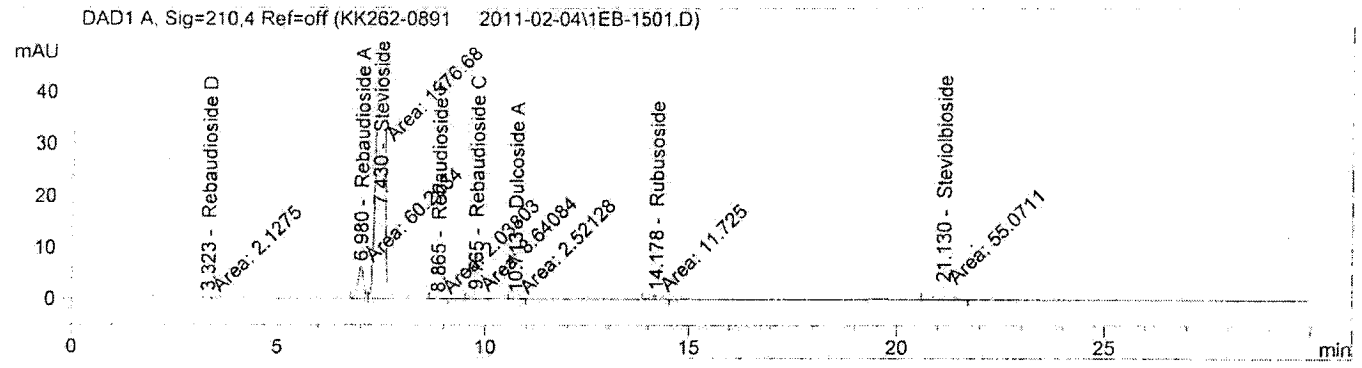
==========

*** End of Report ***

Sample Name: 10-7943

```
================================================================================
Acq. Operator    : Mariel Esguerra                    Seq. Line :  15
Acq. Instrument  : HPLC 10                             Location : P1-E-02
Injection Date   : 2/5/2011 4:32:57 AM                     Inj :  1
                                                     Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\KK262-0891     2011-02-04\KK262.M
Last changed     : 2/4/2011 6:04:26 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\17\DATA\KK262-0891     2011-02-04\KK262.M
Last changed     : 3/18/2011 9:59:38 AM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC JECFA 2010

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\KK262-0891     2011-02-04.SC.SSIzip
ECM Version      : 22
```




```
================================================================================
                          ESTD Percent Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, March 18, 2011 9:50:31 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     1.26200  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           [mAU*s]                  %
-------|------|----------|----------|----------|--|------------------
  3.323 FM        2.12750 1.05263e-3   0.177454    Rebaudioside D
  6.980 MF       60.20337 8.95361e-4   4.271294    Rebaudioside A
  7.430 FM     1576.67859 7.51883e-4  93.936456    Stevioside
  8.865 MF        2.03803 8.72181e-4   0.140850    Rebaudioside F
  9.765 MF        8.64084 8.87218e-4   0.607473    Rebaudioside C
 10.713 FM        2.52128 7.36842e-4   0.147210    Dulcoside A
 14.178 MM       11.72503 6.01504e-3   5.588474    Rubusoside
 19.595             -          -          -        Rebaudioside B
 21.130 MM       55.07109 6.01504e-4   2.624840    Steviolbioside

Totals :                              107.494052


1 Warnings or Errors :
```

000320

Sample Name: 10-7943

Warning : Calibrated compound(s) not found

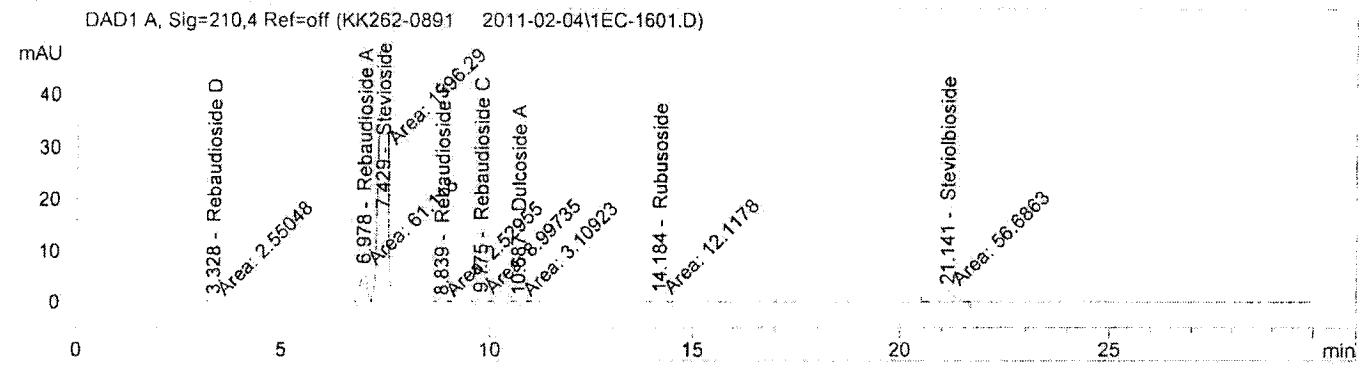
==========

*** End of Report ***

Sample Name: 10-7944

```
==========================================================================
Acq. Operator    : Mariel Esguerra                  Seq. Line :  16
Acq. Instrument  : HPLC 10                           Location : P1-E-03
Injection Date   : 2/5/2011 5:03:45 AM                   Inj :  1
                                                   Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\KK262-0891     2011-02-04\KK262.M
Last changed     : 2/4/2011 6:04:26 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\17\DATA\KK262-0891     2011-02-04\KK262.M
Last changed     : 3/18/2011 9:59:38 AM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC JECFA 2010

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\KK262-0891     2011-02-04.SC.SSIzip
ECM Version      : 22
```




```
==========================================================================
                          ESTD Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, March 18, 2011 9:50:31 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     1.27775  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          [mAU*s]                   %
-------|------|----------|----------|----------|--|------------------
  3.328 FM        2.55048 1.05263e-3   0.210113    Rebaudioside D
  6.978 MF       61.11798 8.95361e-4   4.282734    Rebaudioside A
  7.429 FM     1596.29309 7.51883e-4  93.932764    Stevioside
  8.839 MF        2.52955 8.72181e-4   0.172665    Rebaudioside F
  9.775 MF        8.99735 8.87218e-4   0.624740    Rebaudioside C
 10.687 FM        3.10923 7.36842e-4   0.179301    Dulcoside A
 14.184 MM       12.11782 6.01504e-3   5.704493    Rubusoside
 19.595            -          -          -        Rebaudioside B
 21.141 MM       56.68626 6.01504e-4   2.668520    Steviolbioside

Totals :                              107.775329

1 Warnings or Errors :
```

000322

Sample Name: 10-7944

Warning : Calibrated compound(s) not found

==========

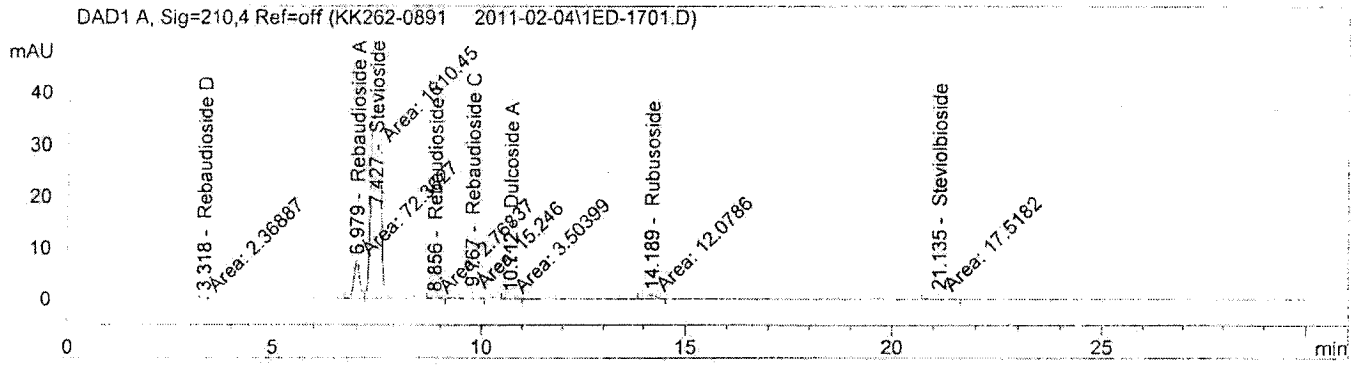
*** End of Report ***

000323

Sample Name: 10-7945

```
================================================================================
Acq. Operator    : Mariel Esguerra                    Seq. Line :  17
Acq. Instrument  : HPLC 10                             Location : P1-E-04
Injection Date   : 2/5/2011 5:34:33 AM                     Inj :   1
                                                     Inj Volume : 5.0 µl
Acq. Method      : C:\CHEM32\10\DATA\KK262-0891      2011-02-04\KK262.M
Last changed     : 2/4/2011 6:04:26 PM by Mariel Esguerra
Analysis Method  : C:\CHEM32\17\DATA\KK262-0891      2011-02-04\KK262.M
Last changed     : 3/18/2011 9:59:38 AM by Mariel Esguerra
Method Info      : Steviol Glycosides by HPLC JECFA 2010

ECM Server       : http://us05sqlc/ecmwg
ECM Operator     : Mariel Esguerra
ECM Path         : Petaluma\LC\HPLC-10\Data\KK262-0891      2011-02-04.SC.SSIzip
ECM Version      : 22
```




```
================================================================================
                          ESTD Percent Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Friday, March 18, 2011 9:50:31 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     1.28100  [mg/mL]
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=210,4 Ref=off
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount %	Grp	Name
3.318	FM	2.36887	1.05263e-3	0.194656		Rebaudioside D
6.979	MF	72.36269	8.95361e-4	5.057823		Rebaudioside A
7.427	FM	1610.44946	7.51883e-4	94.525358		Stevioside
8.856	MM	2.76837	8.72181e-4	0.188487		Rebaudioside F
9.767	MM	15.24597	8.87218e-4	1.055933		Rebaudioside C
10.712	FM	3.50399	7.36842e-4	0.201552		Dulcoside A
14.189	MM	12.07864	6.01504e-3	5.671626		Rubusoside
19.595		-	-	-		Rebaudioside B
21.135	MM	17.51815	6.01504e-4	0.822579		Steviolbioside

Totals : 107.718013

1 Warnings or Errors :

Sample Name: 10-7945

Warning : Calibrated compound(s) not found

==========

*** End of Report ***

APPENDIX C-3

Pesticide Residue Analyses for High Purity Rebaudioside A

Chengdu Wagott Pharmaceutical Co., Ltd.

Mr. Michael Wang
5/F A Building of Tianhe Ave., Hi-Tech Zone,
Fax:0086 28 66070900

SU0000105

Eurofins Tech. Service (Suzhou) Co., Ltd
No. 10 B1, Longshan Road, SND
Suzhou, 215163
Jiangsu Province, P.R.China
Tel: +86 512 69006566
Fax: +86 512 68785966

Date of report: 2011.06.14
Printed by: Lisa Xu

CERTIFICATE OF ANALYSIS

Certificate No.: AR-11-SU-005184-01

Sample

Client Sample Description Stevia Extract (Rebaudioside A 95%)	Sample Code	50211S009043
	Date of order	2011.06.02
	Sample recieved	2011.06.02
	Start of Analysis	2011.06.02
	End of Analysis	2011.06.10
	Reception temperature	25.6℃
	Quantity of Sample	1*100g
	Sample packaging	Sealed aluminum foil bag
	Sample appearance	/
Client sample Code 110403		

Results and comments are shown on the following page(s)

The result(s) relate(s) only to the item (s) tested.
Eurofins General Terms and Conditions apply.

For and on behalf of Eurofins Technology Service (Suzhou) Co., Ltd

000327

(b) (6)

Kevin Yu

Results of Analysis

	Results	LOQ	LOD	MRL	Unit	Comments
SU323	Pesticides herbs, spices, dried products, Quechers, EN 15662, LC-MS/MS					
Screened pesticides	Not Detected	*			*	
SU324	Pesticides herbs, spices, dried products, Internal Method, GC/MS					
Screened pesticides	Not Detected	*			*	

☆ means the test is subcontracted within Eurofins group
⊛ means the test is subcontracted outside Eurofins group

Not Detected: Means not detected at or above the Limit of Quantification (LOQ)
"/" in column MRL means the default MRL value is 0.01 mg/kg
MRL relates to the European regulation

*** A full list of pesticides tested and their LOQ is given on the attached page(s)**

COMMENT

The opinion refers to the tested sample and relates only to the investigated parameters.

Our opinion is that analysed sample is in accordance with the requirements of regulation (EC) 396/2005 (regulation on maximum residue levels in food and feed) in its currently valid version.

We do not accept responsibility for decisions taken on the basis of our reports and opinions.

SU323 Pesticides herbs, spices, dried products LC-MS/MS

analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)
2,4-D	0.01	2,4-D, total		2,4'-Formoxylidid	0.01
3,4,5-Trimethacarb	0.01	3-hydroxycarbofuran	0.01	4-CPA (4-chlorophenoxyacetic acid)	0.01
Abamectin (Sum)		Acephate	0.05	Acetamiprid	0.01
Acibenzolar-s-methyl	0.01	Acifluorfen	0.01	Acrinathrin	0.01
Alachlor	0.05	Aldicarb	0.05	Aldicarb (Sum)	
Aldicarb sulfone	0.01	Aldicarb sulfoxide	0.05	Amitraz	0.01
Amitraz (sum)		Asulam	0.01	Avermectin B1a	0.01
Avermectin B1b	0.01	Azimsulfuron	0.01	Azinphos-methyl	0.05
Azoxystrobin	0.01	Benalaxyl	0.01	Bendiocarb	0.01
Benoxacor	0.01	Bensulfuron methyl	0.01	Bentazone	0.01
Bitertanol	0.01	Boscalid	0.01	Bromoxynil	0.01
Bromuconazole (cis-Isomer)	0.01	Bromuconazole (Sum)		Bromuconazole (trans-Isomer)	0.01
Bupirimate	0.01	Buprofezin	0.01	Butocarboxim (Sum)	
Butocarboxim-sulfoxide	0.01	Butoxycarboxim	0.01	Carbaryl	0.01
Carbendazim (MBC) and benomyl	0.01	Carbofuran	0.01	Carbofuran (Sum)	
Carbosulfan	0.01	Carfentrazone-ethyl	0.01	Chlorfluazuron	0.01
Chloridazon	0.01	Chlorobenzuron	0.01	Chlorpropham	0.01
Chlorpyrifos	0.01	Chlorpyrifos-methyl	0.01	Chromafenoziod	0.05
Clethodim	0.01	Clofentezine	0.01	Clomazone	0.01
Clothianidin	0.01	Cyazofamid	0.01	Cymoxanil	0.02
Cyproconazole	0.01	Cyprodinil	0.01	Cyromazine	0.05
Demeton-S-methyl	0.01	Demeton-S-methylsulfon	0.01	Diazinon	0.01
Diethofencarb	0.01	Diethyl-m-toluamid (DEET)	0.01	Difenoconazole	0.01
Diflubenzuron	0.01	Diflufenican	0.01	Dimethachlor	0.01
Dimethoat/Omethoat (sum of)		Dimethoate	0.01	Dimethomorph	0.01
Diniconazole	0.02	Dinocap	0.01	Disulfoton	0.05
Disulfoton sulfoxide	0.01	Disulfoton-PS-sulfone	0.01	Diuron	0.01
Emamectin (Sum)		Emamectin B1a	0.01	Emamectin B1b	0.02
Epoxiconazole	0.01	Ethiofencarb	0.01	Ethiofencarb (Sum)	
Ethiofencarb-sulfone	0.01	Ethiofencarb-sulfoxide	0.01	Ethoprophos	0.01
Ethoxyquin	0.02	Etofenprox	0.01	Fenarimol	0.01
Fenazaquin	0.01	Fenbuconazole	0.01	Fenhexamid	0.01
Fenobucarb	0.01	Fenoxycarb	0.01	Fenpropimorph	0.01
Fenpyroximate	0.01	Fenthion	0.01	Fenthion (Sum)	
Fenthion-PO-sulfon	0.01	Fenthion-PO-sulfoxide	0.01	Fenthion-PS-sulfon	0.01
Fenthion-PS-sulfoxide	0.01	Fipronil	0.01	Fipronil sulfid	0.01
Fipronil sulfon	0.01	Fluazifop-P-butyl	0.01	Fluazinam	0.01
Fludioxonil	0.01	Flufenoxuron	0.01	Fluopicolide	0.01
Flusilazole	0.01	Flutolanil	0.01	Fomesafen	0.01
Forchlorfenuron	0.01	Formetanate	0.05	Fosthiazate	0.01
Furathiocarb	0.01	Hexaconazole	0.01	Hexaflumuron	0.01
Hexythiazox	0.01	Imazalil	0.01	Imibenconazole	0.01
Imidacloprid	0.01	Indoxacarb	0.02	Iodosulfuron methyl	0.01
Iprodione	0.01	Iprovalicarb	0.01	Isoprocarb	0.01
Isoproturon	0.01	Linuron	0.01	Lufenuron	0.01
Malathion	0.01	Malathion (Sum)		Mepanipyrim	0.01
Metalaxyl	0.01	Metamitron	0.01	Methamidophos	0.02
Methidathion	0.02	Methiocarb	0.01	Methiocarb (Sum)	
Methiocarb sulfone	0.01	Methiocarb sulfoxide	0.01	Methomyl	0.01
Methomyl/Thiodicarb (sum of)		Methoxyfenozid	0.01	Metolachlor	0.01
Metolcarb	0.01	Monocrotophos	0.01	Myclobutanil	0.01
Napropamide	0.01	Neburon	0.01	Nicosulfuron	0.01
Nitenpyram	0.05	Novaluron	0.01	Nuarimol	0.01
Omethoate	0.01	Oxadixyl	0.01	Oxamyl	0.01
Oxamyl-oxime	0.02	Oxydemeton-methyl	0.02	Oxydemeton-methyl + Demeton-S-methyl-sulfon (Sum)	
Penconazole	0.01	Pencycuron	0.01	Pendimethalin	0.01

SU323 Pesticides herbs, spices, dried products LC-MS/MS

analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)
Phorate (Sum)		Phorate Sulfoxide	0.01	Phorate-sulfone	0.01
Phosalone	0.01	Phosmet	0.01	Phoxim	0.01
Piperonyl butoxide	0.01	Pirimicarb	0.01	Pirimicarb (Sum)	
Pirimicarb-desmethyl	0.01	Pirimicarb-Desmethylformamido	0.01	Pirimiphos-methyl	0.01
Primisulfuron-Methyl	0.01	Prochloraz	0.01	Procymidon	0.05
Promecarb	0.01	Propamocarb	0.01	Propargite	0.01
Propham	0.01	Propiconazole	0.01	Propoxur	0.01
Propoxycarbazone	0.01	Propyzamide	0.01	Prosulfocarb	0.01
Prosulfuron	0.01	Pymetrozine	0.05	Pyraclostrobin	0.01
Pyridaben	0.01	Pyrimethanil	0.01	Pyriproxyfen	0.01
Quinoxyfen	0.01	Resmethrin	0.01	Rimsulfuron	0.01
Rotenone	0.01	Sethoxydim	0.01	Simazine	0.01
Simeconazole	0.01	Spinosad		Spinosyn A	0.01
Spinosyn D	0.01	Spirodiclofen	0.01	Spiromesifen	0.01
Spiroxamine	0.01	Tebuconazole	0.01	Tebufenozide	0.01
Tebufenpyrad	0.01	Teflubenzuron	0.01	Tepraloxydim	0.01
Tetraconazole	0.01	Thiabendazole	0.01	Thiacloprid	0.05
Thiamethoxam	0.02	Thiamethoxam (Sum)		Thifensulfuron methyl	0.01
Thiodicarb	0.01	Thiofanox sulfone	0.01	Thiofanox sulfoxide	0.05
Thiophanat-methyl	0.01	Tolclofos-methyl	0.01	Tralkoxydim	0.01
Triadimefon	0.01	Triadimefon and triadimenol		Triadimenol	0.01
Triasulfuron	0.01	Triasulfuron methyl	0.01	Tribenuron-methyl	0.01
Trichlorfon	0.01	Tridemorph	0.01	Trifloxystrobin	0.01
Trifloxysulfuron	0.01	Triflumizole	0.01	Triflumuron	0.01
Triflusulfuron-methyl	0.01	Vamidothion	0.01	Vamidothion-sulfone	0.01
Vamidothion-sulfoxide	0.01	Zoxamide	0.01		

Total parameters 245

SU324 Pesticides herbs, spices, dried products GC/MS

analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)
2-Phenylphenol	0.01	Acetochlor	0.02	Aclonifen	0.05
Aldrin	0.02	Ametryne	0.02	Aramite	0.05
Atrazine	0.02	Benfluralin	0.02	Bifenox	0.05
Bifenthrin	0.01	Biphenyl	0.02	Bromfenvinfos	0.05
Bromophos	0.05	Bromophos-ethyl	0.05	Bromopropylate	0.02
Butachlor	0.01	Butafenacil	0.02	Cadusafos	0.04
Captafol	0.05	Captan	0.05	Carbophenothion	0.05
Carbophenothion-methyl	0.05	Chlorbenside	0.04	Chlordane (Sum)	
Chlordane, alpha	0.01	Chlordane, gamma	0.01	Chlorfenapyr	0.05
Chlorfenson	0.05	Chlorfenvinphos	0.02	Chlormephos	0.05
Chlorobenzilate	0.01	Chloroneb	0.05	Chloropropylate	0.01
Chlorothalonil	0.02	Chlorpyrifos	0.01	Chlorpyrifos-methyl	0.01
Chlorthal-dimethyl	0.01	Chlorthion	0.05	Chlozolinate	0.02
Crufomate	0.02	Cyanazine	0.04	Cyanofenphos	0.02
Cyanophos	0.04	Cyfluthrin	0.05	Cyhalothrin lambda	0.02
Cypermethrin	0.05	Cyphenothrin	0.05	DDD, o,p'-	0.01
DDD, p,p'-	0.01	DDE, o,p'-	0.01	DDE, p,p'-	0.01
DDT (Sum)		DDT, o,p'-	0.01	DDT, p,p'-	0.01
Deltamethrin	0.06	Dichlobenil	0.05	Dichlofenthion	0.02
Dichlofluanid	0.02	Dichlorobenzophenone o,p'	0.02	Dichlorobenzophenone p,p'	0.02
Dichlorvos	0.05	Dicloran	0.05	Dicofol (Sum)	
Dicofol, o,p'-	0.02	Dicofol, p,p'-	0.02	Dieldrin	0.02
Dieldrin (Sum)		Dienochlor	0.05	Dinobuton	0.05
Dioxabenzofos (Salithion)	0.05	Dioxathion	0.05	Diphenylamine	0.02
Edifenphos	0.02	Endosulfan (Sum)		Endosulfan, alpha-	0.05
Endosulfan, beta-	0.05	Endosulfan, sulfat-	0.02	Endrin	0.04
EPN	0.05	Ethalfluralin	0.01	Ethion	0.01

SU324 Pesticides herbs, spices, dried products GC/MS

analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)	analyte	LOQ(mg/kg)
Etridiazole	0.04	Etrimfos	0.02	Famoxadone	0.04
Fenamiphos	0.05	Fenchlorphos	0.02	Fenfluthrin	0.02
Fenitrothion	0.04	Fenpropathrin	0.04	Fenson	0.05
Fenthion	0.04	Fenvalerate & Esfenvalerate (Sum of RS&SR Isomers)		Fenvalerate & Esfenvalerate(Sum of RR&SS Isomers)	
Fenvalerate (RR-/SS)	0.04	Fenvalerate (RS-/SR)	0.04	Fluchloralin	0.02
Flucythrinate	0.05	Flumetralin	0.05	Flumioxazin	0.05
Fluotrimazole	0.05	Fluquinconazole	0.04	Fluvalinate-tau	0.02
Folpet	0.05	Fonofos	0.04	Formothion	0.06
Halfenprox	0.02	HCB	0.01	HCH (Sum, without Lindan)	
HCH gamma(Lindan)	0.02	HCH, alpha-	0.02	HCH, beta-	0.02
HCH, delta-	0.02	HCH, epsilon-	0.02	Heptachlor	0.01
Heptachlor (Sum)		Heptachlor epoxide cis	0.01	Heptachlor epoxide trans	0.02
Heptenophos	0.02	Iprobenfos	0.05	Isazofos	0.04
Isocarbophos	0.04	Isodrin	0.04	Isofenphos	0.04
Isofenphos-methyl	0.01	Isoprothiolane	0.02	Jodfenphos	0.05
Kresoxim-methyl	0.01	Landrin	0.05	Malaoxon	0.05
Malathion (Sum)		Mecarbam	0.04	Mepronil	0.04
Methacrifos	0.02	Methidathion	0.04	Methoxychlor	0.05
Methyl-Pentachlorophenylsulfide	0.01	Metribuzin	0.04	Mevinphos	0.02
Mirex	0.01	Nitrapyrin	0.05	Nitrofen	0.02
Nitrothal-isopropyl	0.02	Octachlorodipropyl ether (S-421)	0.05	Ofurace	0.04
Oxadiazon	0.02	Oxychlordane	0.05	Oxyfluorfen	0.02
Paclobutrazol	0.04	Parathion	0.06	Parathion-methyl	0.04
Pentachloroaniline	0.02	Pentachloroanisole	0.02	Permethrin	0.04
Phenkapton	0.05	Phenothrin	0.04	Phenthoate	0.04
Phorate	0.04	Phosphamidon	0.04	Picoxystrobin	0.04
Piperophos	0.05	Pirimiphos-ethyl	0.01	Procymidone	0.01
Profenofos	0.02	Profluralin	0.02	Prometryn	0.02
Propanil	0.02	Propazine	0.02	Prothiofos	0.05
Pyrazophos	0.02	Pyridalyl	0.04	Pyridaphenthion	0.02
Pyrifenox	0.04	Pyrimethanil	0.01	Quinalphos	0.02
Quintozene	0.02	Quizalofop-P-ethyl	0.04	silafluofen	0.02
Silthiofam	0.02	Tebufenpyrad	0.02	Tecnazene	0.02
Tefluthrin	0.02	Terbufos	0.02	Tetrachlorvinphos	0.02
Tetradifon	0.02	Tetrasul	0.02	Tolylfluanid	0.04
Triallate	0.04	Triazamate	0.04	Triazophos	0.02
Trichloronat	0.02	Trifluralin	0.02	Triticonazole	0.04
Uniconazole	0.02	Vinclozolin	0.02		

Total parameters 194

END OF REPORT

APPENDIX D

Rebaudioside A (95%) Stability Test Data & Result Evaluate

Rebaudioside A （95%）

Stability Test
Data and Result Evaluate

WAGOTT **Laboratory of Chengdu Wagott Pharmaceutical Co., Ltd.**

Content

WAGOTT **Laboratory of Chengdu Wagott Pharmaceutical Co., Ltd.**

I. Apparatus and regents

1. Apparatus

A. HWS temperature and humidity incubator

B. GXZ Light incubator

C. DHG 9140-A Electric drying oven

D. Agilent HPLC

E. CP 225D Electronic Analytical Balance

2. Regent: Acetonitrile (HPLC degree) Phosphate (AR degree)

II. Inspection items and inspection methods

1. Inspection items: Characters, moisture and content

2. Inspection methods: According to the stability testing guidelines of Rebaudioside A(95%)

III. Inspection content

1. Effective factors

Sample of the test: batch number 080902

Inspection conditions: A. High illumination (4500±500LX), B. High temperature (40℃);

C. High humidity (90% RH)

Inspection period: 0, 5, 10 days. The result table followed:

Table 1: Effective factors test for Rebaudioside A(95%)

Inspection Items	Time	Characteristics	RA content %	Total Glycosides %
A. High illumination (4500LX)	0 day	White granular with sweet odor and taste	97.56	100.26
	5 day	White granular with sweet odor and taste	97.44	99.66
	10 day	White granular with sweet odor and taste	97.48	99.68

B. High temperature (40℃)	0 day	White granular with sweet odor and taste	97.56	100.26
	5 day	White granular with sweet odor and taste	97.55	99.83
	10 day	White granular with sweet odor and taste	97.42	99.65
High humidity (90% RH)	0 day	White granular with sweet odor and taste	97.56	100.26
	5 day	White granular with sweet odor and taste	97.49	99.69
	10 day	White granular with sweet odor and taste	97.42	99.60

Conclusion: The high illumination (4500 ± 500LX), high temperature (40℃) high humidity (90% RH) and other environmental conditions showed no significant effects on content of Rebaudioside A and total glycosides of the product.

2. Accelerate stability test

Taking with commercial packaging form, the product was placed at the conditions of temperature (40 ℃ ± 2 ℃) and humidity (75% ± 5% RH) for 6 months, and took sample on the beginning of 0, 1,2,3,6 month. The results see Table 2.

Table 2: Accelerate stability test of Rebaudioside A (95%)

Batch number	Time	Characteristics	Moisture %	RA content %
080901	0 month	White granular with sweet odor and taste	4.47	97.51
	1 month	White granular with sweet odor and taste	4.46	97.62
	2 month	White granular with sweet odor and taste	4.43	97.54

WAGOTT **Laboratory of Chengdu Wagott Pharmaceutical Co., Ltd.**

	3 month	White granular with sweet odor and taste	4.42	97.48
	6 month	White granular with sweet odor and taste	4.46	97.58
080902	0 month	White granular with sweet odor and taste	4.52	97.43
	1 month	White granular with sweet odor and taste	4.54	97.46
	2 month	White granular with sweet odor and taste	4.48	97.38
	3 month	White granular with sweet odor and taste	4.52	97.42
	6 month	White granular with sweet odor and taste	4.50	97.44
081001	0 month	White granular with sweet odor and taste	3.76	97.56
	1 month	White granular with sweet odor and taste	3.78	97.76
	2 month	White granular with sweet odor and taste	3.77	97.62
	3 month	White granular with sweet odor and taste	3.78	97.46
	6 month	White granular with sweet odor and taste	3.83	97.53

Conclusion: The characteristics, moisture and RA content of the product did not show significantly changes when placed in the environment of temperature (40 ℃ ± 2 ℃) and humidity (75% ± 5% RH) within 6 months

3. Long-term stability test

Taking with commercial packaging form, the product was placed at the conditions of temperature (25 ℃ ± 2 ℃) and humidity (60% ± 10% RH) for 6 months, and took sample on the beginning of 0,3,6,9,12,18,24,36 month. The results see Table 3.

Table 3: Long-term stability test of Rebaudioside A (95%)

Batch number	Time	Characteristics	Moisture %	RA content %
080901	0 month	White granular with sweet odor and taste	4.47	97.51
	3 month	White granular with sweet odor and taste	4.44	97.47
	6 month	White granular with sweet odor and taste	4.47	97.56
	9 month	White granular with sweet odor and taste	4.49	97.47
	12 month	White granular with sweet odor and taste	4.48	97.72
	18 month	White granular with sweet odor and taste	4.49	97.34
	24 month			
	36 month			
080902	0 month	White granular with sweet odor and taste	4.52	97.43
	3 month	White granular with sweet odor and taste	4.51	97.62
	6 month	White granular with sweet odor and taste	4.46	97.50
	9 month	White granular with sweet odor and taste	4.49	97.54

WAGOTT **Laboratory of Chengdu Wagott Pharmaceutical Co., Ltd.**

	12 month	White granular with sweet odor and taste	4.48	97.68
	18 month	White granular with sweet odor and taste	4.58	97.46
	24 month			
	36 month			
081001	0 month	White granular with sweet odor and taste	3.76	97.56
	3 month	White granular with sweet odor and taste	3.81	97.53
	6 month	White granular with sweet odor and taste	3.84	97.48
	9 month	White granular with sweet odor and taste	3.82	97.76
	12 month	White granular with sweet odor and taste	3.84	97.86
	18 month	White granular with sweet odor and taste	3.82	97.46
	24 month			
	36 month			

Conclusion: The test is in progress.

APPENDIX E

Summary of Findings from Awney et al. (2010) Study

Summary of Study on Stevioside by Awney et al., 2010[19]

In a recently published exploratory subchronic toxicity study, Awney et al. (2010) investigated the effects of 97% pure stevioside on bodyweight, organ relative weight, hematological and biochemical parameters and enzyme activities in Sprague Dawley. In this 12-week toxicity study, groups of male rats (8/group) were given dirking water containing stevioside. The groups were assigned to drink distilled water (control), low-dose stevioside solution (15 mg/kg/day), high-dose stevioside solution (1500 mg/kg/day) or low-dose stevioside (15 mg/kg/day) plus inulin solution for 12 weeks as the sole source of liquid. Fluid intake was recorded daily and levels of test articles were adjusted weekly to receive the appropriate target concentration. Low dose stevioside (15 mg/kg bw/day) administration without or with inulin for 12 weeks did not reveal any adverse effects on body weight, organs relative weight, hematological and biochemical parameters or enzymes activities. However, treatment with high dose stevioside caused significant changes in several investigated toxicological parameters. Among the hematological parameters, significant changes were noted in all except WBCs, RBCs, and PCV% and in all clinical chemistry parameters except proteins, total lipids, serum alanine aminotransferase (ATL) and aspartate aminotransferase (AST).These data suggest the NOEL of 15mg/kg/day. However, critical review of the publication reveals that the study was poorly designed and implemented. Design deficiencies include: insufficient numbers of animals, group-housing with the potential for stress-related changes, unreliable access to steviol via drinking water resulting in suspect dosing calculations in group-housed cages, no indication of fasting prior to blood collection which affects many chemistry and hematological values, no urine collection and no histopathological evaluations for confirmation of findings beyond the controls. In addition to these study design deficiencies, the report fails to adequately present mean or individual organ weight data and, in general, there appears to be inadequate comparison of study findings against laboratory historical control data. Any one of these oversights could have adversely affected the results and/or interpretation of the hematological and chemistry data.

In addition to the above described parameters, tartrate-resistant alkaline phosphatase (TRAP) levels were measured and found to be significantly decreased (Awney et al., 2010). TRAP is an enzyme that is expressed by bone-resorbing osteoclasts, inflammatory macrophages and dendritic cells. This enzyme was not measured in any previous steviol glycoside studies nor has it been adequately vetted for application in toxicological studies. These investigators did not identify the specific TRAP isomer measured, the methodology employed, the handling of the samples, or any historical data on TRAP levels. The significance and relevance of this poorly documented toxicological endpoint which lacks histopathological confirmation does not appear to have a distinct role in determining the toxicological profile of a material in a test animal. The data presented by Awney et al (2010) are probably not representative of changes due to the subchronic dietary administration of steviol glycoside because of overall poor study design and reliance on the findings of the untested enzyme TRAP. The preponderance of the data from several well designed studies on steviol glycoside suggests that differences noted in hematological and chemistry data are probably random, nonspecific and not toxicologically significant.

[19] Awney, H.A., Massoud, M.I. El-Maghrabi, S., 2010. Long-term feeding effect of stevioside sweetener on some toxicological parameters of growing male rats. Journal of Applied Toxicology, Online Publication: 19 NOV 2010; DOI: 10.1002/jat. 1604.

SUBMISSION END